Development of novel citrate-based dental tissue conditioners

Thesis submitted in partial fulfilment of the requirements of the Degree of Doctor of Philosophy

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In the Name of God, The Most Gracious, Most Merciful

Dedicated to my Wife and Parents

Abstract

Dental tissue conditioners are compliant, viscoelastic gels used primarily to form a soft cushion between the oral mucosa and the hard denture base. Their uses include the treatment of inflamed mucosa resulting from ill-fitting dentures and in treatment of denture related stomatitis. They are presented in powder/liquid format where the powder is usually poly(ethyl methacrylate) (PEMA) and the liquid is a mix of an aromatic ester (plasticiser, usually a phthalate) with ethanol. In use, the ethanol and plasticiser leach out with time causing the material to harden. In recent years there has been concern about possible toxic effects of the leached phthalate. Preliminary work has shown citrate plasticisers to be acceptable replacements for phthalates.

Another disadvantage of the powder/liquid format is the porosity produced on mixing which can lead to microbial ingress and contamination. One possible solution would be to use a pre-gelled material which would have the advantages of easy application and reduced porosity.

Candidal infections are a common etiological factor in denture related stomatitis. Earlier studies have shown it possible to release chlorhexidine diacetate (a broad spectrum antibacterial/antifungal agent) from powder/liquid tissue conditioners to treat these infections

The aim of this study is to develop citrate-based pre-gelled and powder/liquid tissue conditioners and explore its use as potential drug delivery vehicle for chlorhexidine diacetate.

The experimental pre-gelled system (EPGS) containing PEMA and acetyl tributyl citrate (ATBC) only showed stable Shore A hardness values over an 18 month time

period when stored at 7°C. The Shore A hardness and creep compliance ratio (flow) of EPGS indicated that it could be used as both a tissue conditioner and a temporary denture lining material, whereas experimental powder liquid system (EPLS), which contained 16 hours ball-milled PEMA powder and ATBC plus 5% ethanol, had more suitable properties for use as a tissue conditioner. Addition of chlorhexidine diacetate alone or with sodium fluoride did have an effect on the hardness and creep compliance ratio of the materials but these were within acceptable range. Both EPGS and EPLS containing 1% chlorhexidine had a higher percent release than those containing 9% chlorhexidine. The addition of sodium fluoride increased the release of chlorhexidine in all formulations.

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

Acetyl triethyl citrate	ATEC
Acetyl tri-n-butyl citrate	ATBC
Acetyl tri-n-hexyl citrate	ATHC
Artificial saliva	AS
Benzyl benzoate	BB
Benzyl salicylate	BS
Butyl phthalyl butyl glycollate	BPBG
Butyryl tri-n-hexyl citrate	BTHC
Chlorhexidine diacetate	CHD
Controlled drug delivery	CDD
Creep compliance ratio	CCR
Desorption diffusion coefficient	D_{des}
dibutyl sebecate	DBS
di-n-butyl phthalate	DBP
Distilled water	DW
Experimental powder liquid system	EPLS
Experimental pre-gelled system	EPGS
Glass Transition Temperature	T_g
Hydroxyethylmethacrylate	HEMA
International organization for standardisation	ISO
International rubber hardness degree	IRHD
Molecular weights	M_w
n-butyl/ethyl methacrylate	BMA/EMA
Percent	%
Poly ethyl methacrylate/tetrahydrofurfuryl methacrylate	PEM/THFM
Poly(ethyl methacrylate)	PEMA
Poly(methyl methacrylate)	PMMA
Powder liquid	P/L
Sodium Fluoride	NaF
Solubility parameter	δ
Standard deviation	SD
Tributyl citrate	TBC
Triethyl citrate	TEC

Triethyleneglycol dimethacrylate	TEGDMA
Ultra violet/visible	UV/vis
Urethane dimethacrylate	UDMA
Viscogel old formulation	VG Old
Volume by volume	v/v
Weight by weight	w/w

List of Commercial Soft Liners & Tissue Conditioners

Brand Name	**Manufacturers**
Acrosoft	Acropars, Iran
Caulk Lynal (CL)	L.D. Caulk Co.
Coe –Soft (CS)	GC America Inc.
Coe-Comfort (CC)	Coe lab. Inc.
Denture Soft II (DS)	Chem. Ins. Co. Ltd.
Dentusoft	Densell, Dental Medrano
Dinabase	Medident Lab.
Eversoft	Myerson, Austenal Ltd.
Fit Softer (FS)	Dentsply-Sankin Co.
GC-Soft Liner (GC)	GC Dental Industry Co.
Hydro-Cast (HC)	K.C. Dental Mfg. Co.
Kerr FITT (KF)	Kerr Group
Snug	Mentholatam®
Soft Conditioner (SC)	GC Co.
Softtone	Bosworth Company
Tempo	Lang Dental Mfg. Co., Inc.
Tissue Care	Tokuyama
Tissue conditioner (TC)	GC Co.
Tissue Conditioner II	Shofu Inc.
Treatment Liner	H.D. Justi & Sons, Inc.
Truesoft	Bosworth Company
Veltec	Teledyne Getz
Vertex	Dentimex BV
Viscogel (VG)	Dentsply Ltd.

CHAPTER ONE: INTRODUCTION

1 Introduction

Dental tissue conditioners are viscoelastic gels which are mainly used to form a soft cushion between the tissue surface of the hard denture base and oral mucosa. They are used when there is an underlying problem with the oral mucosa due to poor oral hygiene, ill-fitting dentures, resorption of the alveolar ridge that can lead to inflammation, ulcerations and sometimes to treat fungal infection caused by *Candida albicans*. They help to redistribute the forces evenly on the oral mucosa to promote healing. In short they are used as temporary relining materials for immediate dentures, in the treatment of denture related stomatitis where candidal infection is a common etiological factor, as a functional impression material and in some prosthetic devices e.g. obturators.

They are presented as a powder/liquid system ready to be mixed and used. The powder is most commonly poly(ethyl methacrylate), or a related copolymer; whereas the liquid consists of a mixture of a plasticiser, usually an aromatic ester, and ethanol as solvent. After mixing, the ethanol swells the polymer powder allowing penetration of the plasticiser. The polymer chains become more mobile and a gel is formed by polymer chain entanglement, which is a physical reaction not a chemical one. The rate of gelation depends upon a number different aspects of composition; molecular weight and particle size of polymer powder, molar volume of plasticiser, amount of ethanol and powder/liquid ratio.

The problems associated with current tissue conditioners available on the market are described subsequently. Tissue conditioners become hard in the oral cavity initially due to the leaching of ethanol and then plasticiser. The rate of plasticiser leaching is affected by its molecular weight, molar volume, water solubility and the amount of ethanol in the formulation. Methods used to reduce plasticiser leaching such as using higher molecular weight plasticiser, reducing ethanol amount etc., also increase the gelation time to an unacceptable level to be used as a chair side material in the clinics (Jones *et al.*, 1986; Parker and Braden, 1990).

In recent years there has been concern about possible toxic effects of the leached phthalate plasticisers, commonly used in tissue conditioners. They are believed to have possible cytotoxic, carcinogenic and oestrogenic activity (Okita and Hensten-Pettersen, 1991; Hashimoto *et al.*, 2003). Hence, citrate-based plasticisers have been used as suitable replacements.

Tissue conditioners are most commonly available as powder to liquid systems with different compositions. Practically, altering the powder to liquid ratio (factor controlled by the clinicians) affects the properties of these materials. Another disadvantage of the powder/liquid systems is the resulting porosity produced, which can lead to microbial ingress and contamination (Wright, 1980).

One possible solution to overcome the problems associated with tissue conditioners is to develop a citrate based pre-gelled tissue conditioner formulation. This will result in a standardised gel with optimal properties, ease of use at the chairside, a reduction or elimination of air bubbles thus improving the hygiene of the dentures

and as a controlled drug delivery vehicle for treatment of Candidal infections, which is commonly seen in denture wearers.

Hence one of the aims of this research was to develop a pre-gelled tissue conditioner incorporating a citrate plasticiser and no ethanol. Furthermore, the physical properties of an experimental pre-gelled formulation were compared with experimental and commercial powder/liquid formulations. Viscogel and Coe-Comfort were selected as commercial materials for this study due to represent the range of commercial tissue conditioners available, where Viscogel is recommended for general applications and Coe-Comfort mainly as a tissue conditioner.

When developing a new tissue conditioner formulation, it is important to have knowledge of its basic properties that are required for its clinical use. These properties include gelation time, water uptake behaviour, hardness and creep compliance (flow properties). Gelation time is important as these materials are used at the chairside and therefore a short gelation time is desirable. However with a pre-gelled system this is not important. Due to these materials being used in an aqueous environment it is essential to study their water uptake behaviour and their interaction with the environment in which they are used. Similarly hardness and creep define the materials usage in a particular clinical situation.

Viscogel as commercial control together with experimental P/L and pre-gelled formulations could be investigated further as drug delivery vehicles for the potential treatment of Candidal infections. Therefore it was decided to add chlorhexidine diacetate, as an anti-fungal drug, with and without the addition of 0.5% NaF to these

materials and study their water uptake and chlorhexidine diacetate/fluoride release. The effect of incorporating chlorhexidine and NaF on the physical properties of the formulations, e.g. Shore A hardness, creep compliance ratio and gelation time (for powder/liquid formulations), were also studied.

CHAPTER TWO: LITERATURE REVIEW

2 Literature Review

2.1 Denture Lining Materials

The alveolar ridge changes with the use of dentures and sometimes it is necessary to make changes to the fit surface of the acrylic dentures in order to improve adaptation to the oral tissues. So either the whole of the denture base is replaced with heat-cured acrylic resin or a self-cured resin is applied to the fitting surface of the existing base. Occasionally it is necessary to apply a very soft material to the tissue surface of the denture, to provide a cushioning effect to traumatized soft tissue, to allow recovery before recording an impression for a new denture. Some denture wearers cannot tolerate a hard denture base so a long term soft cushion is applied on the fitting surface of the dentures. These materials can be classified into three groups (McCabe and Walls, 2008; Parker and Braden, 1982; Braden *et al.*, 1997)

1. Hard reline materials
2. Soft lining materials
    a. Long-term soft lining material
    b. Temporary soft lining material
3. Tissue Conditioners

Hard reline materials are used to replace the tissue surface of an existing denture. This requires taking an impression of the soft tissues using the denture as an impression tray and the relining is carried out in a laboratory with a heat cured material (McCabe and Walls, 2008).

Soft lining materials are much softer than the conventional denture base acrylics, as the name indicates. They are divided into two types based on their usage. Long-term soft lining materials are meant for those patients who cannot tolerate a hard denture base. This is mostly because of the irregular alveolar ridge (usually mandibular) which is covered by non-resilient and thin mucosa. Long-term soft lining materials provide relief to the pain caused by the masticatory loads (through a hard base) in these patients; they are intended to last for the life time of the dentures. They are normally applied at the time of production of a new denture in a laboratory (McCabe and Walls, 2008).

The temporary soft lining materials are intended to be used for a few months as they have a limited life. They are room temperature cured chair side materials that are directly applied on the denture base by the dentist (McCabe and Walls, 2008). Currently available soft lining materials can be classified according to the types of materials as shown in Figure 2.1 (Qudah *et al.*, 1990; McCabe and Walls, 2008):

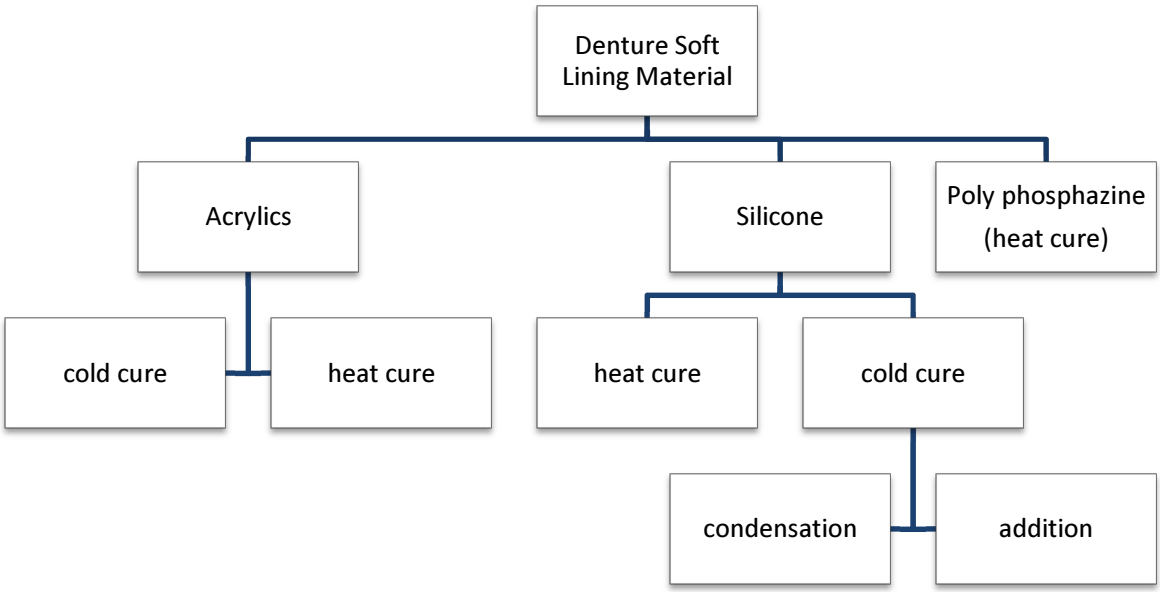

Figure 2.1: Different types of Soft Lining Materials

Tissue conditioners and temporary soft relining materials have similar properties and are often referred to as similar materials in literature. The difference between the two is that temporary soft lining materials are set by a polymerisation reaction and will last several weeks in the mouth whereas tissue conditioners gel by polymer chain entanglement only and require replacement after two to three days (Braden *et al.*, 1997).

2.2 Tissue Conditioners

Tissue conditioners were first proposed by Chase in 1961 and described by Smith in 1962, as temporary variants of the permanent liners that were already in use at that time (Chase, 1961; Smith, 1962). Tissue conditioners are commonly composed of an acrylic polymer e.g. poly(ethyl methacrylate) (PEMA) or a copolymer powder e.g. n-butyl methacrylate/ethyl methacrylate (BMA/EMA) and a liquid consisting of

plasticiser (commonly a phthalate) and ethanol as a solvent. Table 2.1 shows typical compositions of some commercially available tissue conditioners.

Table 2.1 Composition of some commercial tissue conditioners (Jepson *et al.*, 2000)

Materials	Polymer	Plasticiser	Solvent
Coe-Comfort	PEMA	Benzyl Benzoate (87.3%) Di-n-butyl phthalate (4.5%)	Ethanol (8.2%)
Coe Soft	PEMA	Benzyl salicylate (35.1%) Di-n-butyl phthalate (49.7%)	Ethanol (15.2%)
Soft-Liner	PEMA	Butyl phthalyl butyl glycollate (80.9%) Di-n-butyl phthalate (4.3%)	Ethanol (14.8%)
Viscogel (VG)	PEMA (86.2%) Poly(methyl methacrylate)(13.8%)	Butyl phthalyl butyl glycollate (86.9%) Di-n-butyl phthalate (8.2%)	Ethanol (4.9%)

Coe-Comfort (Coe lab Inc.), Coe Soft (GC America), Soft-Liner (GC Dental Industry), Viscogel (Dentsply)

They are usually available in a powder and liquid (P/L) form, however similar materials are also available as over-the-counter products in the form of sheets or as a gel in tubes. Some common brand names are Snug (Mentholatam®) and Dinabase (Medident Lab.).

Tissue conditioners are temporary materials used in denture wearers. When the powder and liquid are mixed a gel is formed. This gel is viscoelastic in nature responding elastically to the rapid dynamic loading of masticatory forces, but will flow under constant masticatory loads (Braden *et al.*, 1997). Tissue conditioners harden with time in the oral cavity due to the leaching of ethanol initially and

subsequently as a result of loss of plasticiser (Braden and Causton, 1971). They should be replaced after every 2-3 days when used as tissue conditioners by the dentist, up to 3 months usage as a temporary relining material and after 24 hours as a functional impression material (Graham *et al.*, 1991b).

Home reliners are supplied in pharmacies as a paste packed into a tube to be used by the patients. They are often used to provide cushioning effect from ill-fitting dentures (Hirayama *et al.*, 2015). They are soft materials and mainly comprised of polyvinyl acetate containing varying amounts of ethanol (Takahashi, 2003). The polyvinyl acetate is amorphous polymer and in addition they also include calcium carbonate, polypropylene glycol, white bees wax and alkyl methacrylate copolymers (Murata *et al.*, 2010; Prasad *et al.*, 2014). Home reliners are not very popular among the dentists because of their poor properties (Woelfel and Kreider, 1968; Means, 1964). In a recent study on the physical properties of denture home reliners by Murata *et al.* (2010) it was found that they lack the cushioning effect and their softness is lost very quickly, usually within a day. This is because of the presence of large amounts of ethanol they contain, which can be as high as 40%. The authors believed that ethanol leaches out very quickly and is responsible for the quick loss of softness and large water uptake in these materials. In contrast to this study Udo-Yamakawa and Kawai (2010) suggested that home reliners can be temporarily used effectively under professional care. Home reliners are generally abused by the patients and are not recommended by the dentists (Hirayama *et al.*, 2015) however; there are few scientific studies that have been conducted on the properties and effects of the home reliners.

2.3 Uses of Tissue Conditioners

2.3.1 Mechanical Trauma

Stresses produced in the mouth are transferred to the supporting underline bone through oral mucosa which acts as a cushion. If the denture does not fit properly then the soft tissues can experience excessive forces, resulting in tissue damage that ranges from slight displacement to gross deformation. In severe cases there is generalized redness and swelling. Tissue conditioners can be applied to the fitting surface of the denture to equalize distribution of occlusal forces by providing a cushion on the mucosa thus permitting the healing of the inflamed tissue. The tissue should recover sufficiently in a week so that a new denture can be made, but in severe cases further application may be required (Harrison, 1981).

2.3.2 After Care of Immediate Dentures

Tissue conditioners can be used as a temporary soft lining on the fitting surfaces of immediate dentures following extraction. This results in reduction of both post-operative pain and denture problems because of their compliant viscoelastic nature (Hopkins, 1979).

2.3.3 In Treatment of Denture Stomatitis

Tissue Conditioners are commonly used for the treatment of denture related stomatitis (Prasad *et al.*, 2014). Denture stomatitis is also known as denture sore mouth and chronic atrophic candidiasis. It consists of a mild inflammation and erythema of the mucosa beneath the denture that occurs mostly in a complete upper

denture (Scully, 2008). It is usually asymptomatic but patients may complain of mucosal bleeding, swelling, burning sensation, halitosis, unpleasant taste and dryness of the mouth. About 70% of the denture wearers suffer from this condition (Arendorf and Walker, 1987). It is slightly more prevalent in females than males (Mikkonen *et al.*, 1984).

2.3.3.1 Predisposing Factors of Denture Stomatitis

Denture stomatitis is caused by multiple factors. These factors include denture trauma, poor oral and denture hygiene, denture wearing especially throughout night or with a dry mouth, fungal infection, hypersensitivity to denture base material, smoking, diabetes or high carbohydrate diet and HIV (rare factor) (Wilson, 1998; Figueiral *et al.*, 2007)

2.3.3.2 Clinical Features and Classification of Denture Stomatitis

Denture stomatitis is presented with chronic erythema and oedema of the mucosa that comes in contact with the fitting surface of the denture (mostly upper complete denture), which is restricted to the denture bearing area. It is usually asymptomatic and uncommon complications include angular stomatitis and papillary hyperplasia in the vault of the palate (Figueiral *et al.*, 2007).

Diagnosis is usually made on the clinical symptoms. It is classified into three different types increasing in severity (Newton, 1962):

- Type 1: a localised simple inflammation or a pinpoint hyperaemia
- Type 2: an erythematous or generalized simple type, presented as a more diffuse erythema, involving a part of, or the entire, denture covered mucosa
- Type 3: a granular type (inflammatory papillary hyperplasia) commonly involving the central part of the hard palate and the alveolar ridge.

2.3.3.3 Treatment of Denture Stomatitis

Treatment of denture stomatitis includes improvement in oral and denture hygiene, correction of denture faults, relieving of stresses on the mucosa by tissue conditioners and prescription of antifungal drugs. Although several methods are used for treatment, there are three major approaches used widely which are (Wilson, 1998; Chow *et al.*, 1999; Uludamar *et al.*, 2010):

1. Effective cleaning of dentures using denture cleansers.
2. Concentration of the treatment with antifungal drugs on the palate.
3. Replacing the dentures with new ones or using tissue conditioners to reduce the trauma.

Tissue conditioners have been studied as potential drug delivery vehicles, to deliver anti-fungal drugs directly at the site of infection (Sample, 2001; Geerts *et al.*, 2008; Radnai *et al.*, 2009; Falah-Tafti *et al.*, 2010; Urban *et al.*, 2014). This role is further discussed in the drug delivery section 2.7.3.

2.3.4 As Functional Impression

According to Razek (1979) “a functional impression material is one which is applied to the basal surface of a denture to make an impression under functional stress”. It has been found that tissue conditioners are useful in taking a functional impression of the edentulous arch where they are kept in the mouth for 24 hours (Razek, 1979; Harrison, 1981; McCarthy and Moser, 1978).

2.3.5 Other Uses

Tissue conditioners have also been used in a variety of different applications due to their compliant viscoelastic properties. They include (Frisch *et al.*, 1968; McCarthy and Moser, 1978; Loh and Tan, 1986):

- As immediate surgical splints
- As stents for haemophiliacs
- As post-surgical periodontal packs
- As obturators for surgical defects resulting from oral cancer removals
- In cleft palate defects to aid in the speech

2.4 Requirements of Tissue Conditioners

International Organisation for Standardisation (ISO) defines some minimum and desirable requirements for the long term and temporary lining materials (ISO 10139-1, 2005 and ISO 10139-2, 2009 respectively; ISO, 2009) .

The requirements for a tissue conditioner are as follows:

1. It should be biocompatible i.e. nontoxic and non-irritant to both patient and dental staff.
2. It should be tasteless and odourless.
3. It should be quickly and easily processable using conventional dental techniques.
4. It should be easy to clean and should be stable in the oral environment, especially in the presence of food, drinks or tobacco. Also denture cleansers should not have an effect on the lining as it has been found that these can have a dramatic effect on the mechanical properties of the materials leading to clinical failure (Braden *et al.*, 1995).
5. There should be no voids or porosity as these may be potential sites for microbial growth.
6. The material should be dimensionally stable during its processing and use in the mouth. The water absorption should be low as high water absorption leads to swelling of the material resulting in dimensional change. This may lead to straining of the denture base interface, increase distortion and reduce bonding (Braden and Causton, 1971). Swelling also allows the ingrowth of microbial organisms.
7. The material should not support the growth of microbial organisms especially *C. albicans* (Wright, 1980). *C. albicans* is part of the natural flora of the mouth and is a common etiological factor for denture related stomatitis (Arendorf and Walker, 1987).
8. There should not be any leaching of constituents from the material, however if it does occur, it should be minimal.
9. The material should have adequate mechanical properties so that it can perform its purpose adequately and effectively i.e. it should have appropriate

viscoelastic properties and a low modulus but sufficient to withstand the masticatory forces. It should also maintain good adaptability to the contours of the ridges in the mouth so that its cushioning effect is maintained.

10. The material should adhere well to the denture base to provide good stability. If there is weak bonding between the two materials then there may be separation of the two which may lead to poor hygiene due to difficulties in cleaning the denture.
11. It should have good wettability so that a thin layer of saliva is formed between alveolar ridges and polymer base. This helps in lubrication of the surfaces resulting in better comfort and retention of the denture. Poor wettability may lead to increase in friction and thus tissue damage (Wright, 1981).

Every material has some short comings and this is also true of current tissue conditioners currently available on the market when comparing their properties with the ideal properties. There are concerns regarding the biocompatibility of the constituents leached out of the materials. Most of the materials are in powder liquid format and mixing is done by a dentist or dental assistant. Although some manufacturers include measuring cups for dispensing powder and liquid, however the standardization of the correct ratio for mixing is difficult to achieve due to human errors which can lead to a material which does not have optimal properties. Mixing produces air bubbles which can be a potential site of growth for microbial organisms. Tissue conditioners are used in a moist environment so water absorption occurs in different amounts as each commercial material has different composition. The effects of these on different properties are discussed further in the literature review.

ISO 10139-2:2009 has classified the soft lining materials as “soft” if Shore A hardness is 25-50 and “extra soft” if the Shore A hardness is ≤ 25. Similarly the penetration ratio (R) i.e. the penetration at 30sec and 5sec determines the resistance to flow. Class I is high resistance to flow if R ≤ 1.1 and class II is low resistance to flow if 1.1 < R ≤1.75 (McCabe and Walls, 2008). Clinically tissue conditioners are used as temporary denture lining materials, as tissue conditioning materials and as functional impression materials. The optimal properties required in each clinical situation are summarized in Table 2.2 (Murata *et al.*, 1996; Craig, 1997; Gonzalez, 1977; McCabe and Walls, 2008).

Table 2.2: Properties of tissue conditioners required in clinical uses

Clinical Use	Tissue Conditioning	Temporary Denture Lining	Functional Impression
Properties Required	Hardness 13-49 Penetration (R) Ratio: 1.1<R<1.75	Hardness 20-25 Penetration (R) Ratio: R≤1.1	Relatively low hardness* Relatively high flow*

*There are no specific values recommended for tissue conditioners as functional impression in the literature

2.5 Composition of Tissue Conditioners

As mentioned in section 2.2 tissue conditioners are composed of polymer powder and a liquid that contains plasticiser and ethanol.

2.5.1 Polymer Powder

Poly(ethyl methacrylate) PEMA (Figure 2.2) (Braden, 1970a) or a copolymer (e.g. n-butyl methacrylate/ethyl methacrylate, BMA/EMA) (Parker and Braden, 1990), are the most common polymers used in tissue conditioners. The type and class of polymer powder, its molecular weight, size and shape of polymer particles, affects the gelation process of tissue conditioners.

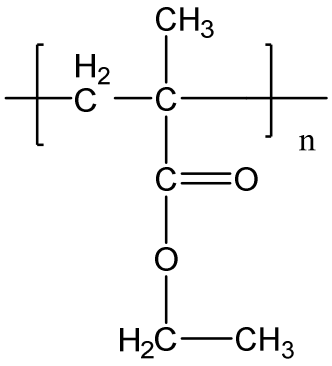

Figure 2.2: Chemical Structure of PEMA polymer

Glass transition temperature (T_g) of polymer powders can indicate which types of polymer powders are suitable for use in tissue conditioner formulations. T_g is the temperature where there is a sudden change in the physical properties especially elastic modulus of the polymer. At the T_g there is a clear change from brittle to ductile behaviour (Anusavice *et al.*, 2012), where the material is brittle below its T_g and rubbery or ductile above it. It is necessary for a tissue conditioner to possess a T_g below mouth temperature so that it is sufficiently compliant for its intended purpose. Plasticisers are added to the monomers/polymers to lower the T_g of the resulting polymers. Therefore, a large amount of plasticiser would be required to lower the T_g of poly(methacrylates) to below mouth temperature compared to PEMA

(Jones *et al.*, 1991), as seen by the T_g values of some poly alkyl methacrylates listed in Table 2.3 (McCabe, 1976). For use as tissue conditioners, polymers that have lower T_g values than ethyl methacrylate, are so soft at room temperature that their fine particles coalesce, and are therefore not conveniently dispensed in powder form (Table 2.3). So the choice of using a polymer powder is limited to those which form free running powders at room temperature (T_g above room temperature), but do not require too much of plasticiser for lowering the T_g to below mouth temperature. PEMA or a copolymer would be the best choice to use (McCabe, 1976; Braden *et al.*, 1997).

Table 2.3: Glass transition temperature of some methacrylate polymers and copolymers (McCabe, 1976; Parker and Braden, 1990)

Polymers (methacrylates)	T_g (^oC)
Methyl	105
Ethyl	65
Propyl	35
n-Butyl	20
Isobutyl	70
80/20 n-butyl/ethyl	37.3
60/40 n-butyl/ethyl	40
50/50 n-butyl/ethyl	46.3

Solubility parameters (δ**)** are also an important factor in the use of PEMA in tissue conditioners. PEMA has δ values in the range of the alcohols listed in Table 2.4. The latter swell the polymer beads whereas they are unlikely to do so in PMMA. Poly (n-butyl methacrylate) has a similar δ to PEMA but it's very low Tg limits its use as it

does not form a free-running powder. Also from the δ values for the plasticisers, PEMA is a much better choice as its δ value is in a similar range (Table 2.4).

Table 2.4: Solubility parameters of Tissue Conditioner components (Jones *et al.*, 1986; Parker and Braden, 1990; Murata *et al.*, 2005)

Polymer	δ $(J/m^3)^{½}$	Plasticiser	δ $(J/m^3)^{½}$
Poly (methyl methacrylate)	0	Benzyl salicylate (BS)	22.54
Poly (ethyl methacrylate)	19.4-23.3	Acetyl tributyl citrate (ATBC)	18.74
Poly (n-butyl methacrylate)	15.1-22.7	Acetyl trihexyl citrate (ATHC)	18.21
Alcohol		Acetyl triethyl citrate (ATEC)	19.19
Ethanol	26.0	Tributyl citrate (TBC)	19.01
n-Hexanol	21.9	Triethyl citrate (TEC)	19.32
n-Octanol	21.1	Dibutyl sebacate (DBS)	17.8
Cetyl alcohol	16.2	Dibutyl phthalate (DBP)	19.2
		Benzyl benzoate (BB)	21.98
		Butyl benzyl phthalate (BBP)	18.67
		Butyl phthalyl butyl glycollate (BPBG)	21.56

The choice of the type of polymer or copolymer used depends not only upon the T_g but also on the rate of absorption of the solvent. PEMA (or copolymers based on this polymer) give reasonable products because of the speed of dissolution in ethanol; poly (methyl methacrylate) (PMMA) is not suitable because it is only swollen (not dissolved) slowly by ethanol. So PEMA or related copolymers are the most suitable

materials in producing tissue conditioners (Braden, 1970a). The role of polymer powder in tissue conditioners is further discussed in section 2.6.

2.5.2 Plasticisers

Plasticisers are generally described as substances added into a material to increase the flexibility, workability and softness of the material. Plasticisers are typically high boiling, oily organic liquids which are usually colourless and odourless (ECPI, 1999).

Plasticisers penetrate between polymer chains, reducing the intermolecular forces, so that the individual chains can slip past one another more easily. Their use in tissue conditioners allow gelation to occur by polymer chain entanglement producing a soft gel that is able to provide a cushioning effect (Braden, 1970a; Jones *et al.*, 1986).

As plasticisers are not bound chemically to the polymer, they leach out of the tissue conditioner during usage in the mouth (Wilson, 1995). As a result of this, they gradually become hard and need to be replaced regularly and so have a limited lifetime in the mouth (Jones *et al.*, 1988).

Plasticisers currently commonly used in tissue conditioners can generally be divided into two different groups i.e. phthalates and citrates, which will be discussed below. Other plasticisers are also used including benzyl salicylate (BS), benzyl benzoate (BB), and dibutyl sebecate (DBS) (Murata *et al.*, 1997; Murata *et al.*, 1993).

2.5.2.1 Phthalate Plasticisers

Phthalates are esters of phthalic acid and are commonly used in the plastic industry. They are used in many daily products ranging from footwear, electric cables, stationary, toys to life saving medical devices e.g. medical tubing and blood bags (ECPI, 1999). Many commercial tissue conditioners contain di-n-butyl phthalate (DBP), butyl phthalyl butyl glycollate (BPBG) (Figure 2.3), and butyl benzyl phthalate (BBP) as plasticisers (Jones *et al.*, 1988; Murata *et al.*, 1997). Table 2.5 shows some commonly used plasticisers in commercial tissue conditioner formulations.

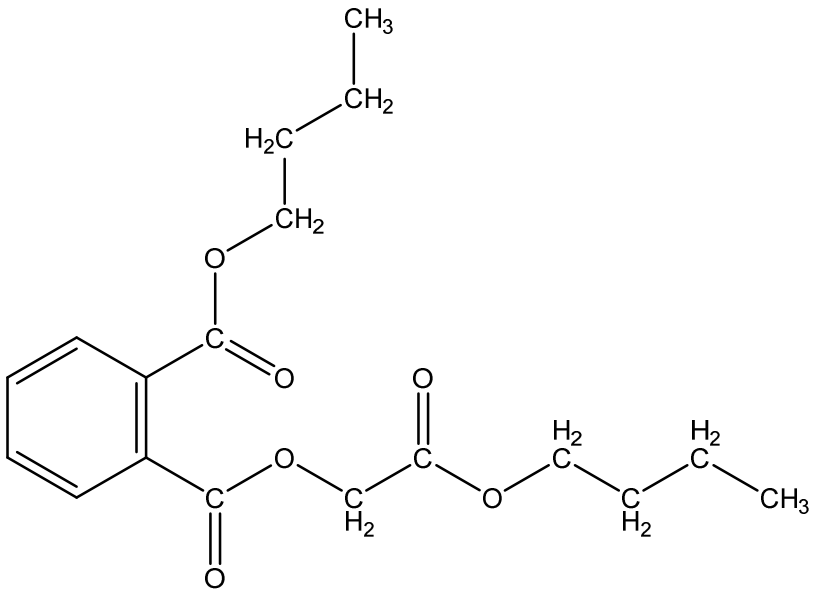

Figure 2.3: Chemical Structure of Butyl phthalyl butyl glycollate

Table 2.5: Some commonly used plasticisers in tissue conditioners (Jones *et al.*, 1986; Takamata *et al.*, 2007; Jepson *et al.*, 2000)

Plasticisers	Abbreviation	Commercial Tissue Conditioners	Molecular Weight (g/mol)
butyl phthalyl butyl glycolate	BPBG	VG, FS, DS, GC and CL	336
butyl benzyl phthalate	BBP	HC and TC	312
di butyl phthalate	DBP	CC, CS, GC,VG	278
benzyl salicylate	BS	CS	228
Benzyl benzoate	BB	CC	212

Viscogel (VG; Dentsply), Fit Softer (FS; Dentsply-Sankin), Denture Soft II (DS; Chem. Ins Co), GC-Soft Liner (GC; GC Dental Industry), Caulk Lynal (CL; L.D. Coulk), Hydro Cast (HC; K.C Dental Mfg Co), Tissue conditioner (TC; GC Co), Coe-Comfort (CC; Coe lab Inc), Coe-Soft (CS; GC America)

One of the main issues is the leaching of phthalates from the material which is further discussed in section 2.6.4.2, giving rise to concerns with the biocompatibility which is discussed further in section 2.5.2.3.

2.5.2.2 Citrate Plasticisers

Citrate esters are all derived from citric acid, a tribasic monohydroxy acid that is found naturally in citrus fruits. Citric acid is commonly used as a flavouring agent, in several food and beverage products like pudding, cake mixes and soft drinks (Frank, 2005). They are made by esterification and acetylation of long chains of esters where the hydroxyl group is reacted to form acetyl or butyryl derivatives (Wilson, 1995). Generic formula for the citrate esters is shown in Figure 2.4 and some commonly used citrates are given in Table 2.6.

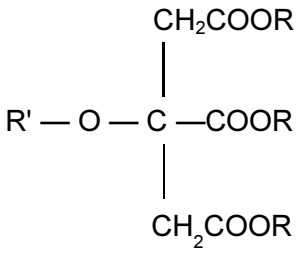

Figure 2.4: Generic formula for the citrate esters (R and R' are defined in Table 2.6)

Table 2.6: Some commonly used Citrate plasticisers (Morflex, 1996)

Generic Name	Abbreviation	R'	R	Molecular Weight (g/mol)
Triethyl Citrate	TEC	H	Ethyl	276
Acetyltriethyl Citrate	ATEC	OAc*	Ethyl	318
Tri-n-butyl Citrate	TBC	H	n-Butyl	360
Acetyltri-n-butyl Citrate	ATBC	OAc*	n-Butyl	402
Acetyltri-n-hexyl Citrate	ATHC	OAc*	n-Hexyl	486
n-Butyrltri-n-hexyl Citrate	BTHC	OBu**	n-Hexyl	514

* OAc = Acetate (CH_3COO^-), ** OBu = Butyrate ($C_4H_7O_2^-$)

Among other citrate plasticisers, acetyltri-n-butyl citrate (ATBC) has been shown to be safe by the U.S. Food and Drug Administration (Lehman, 1951). ATBC, Acetyltri-n-hexyl citrate (ATHC) and n-Butyryltri-n-hexyl citrate (BTHC) are used in medical devices (Morflex, 1996). ATBC is specifically recommended for medical devices and other sensitive applications whereas ATHC and BTHC are intended primarily for use as plasticisers for medical plastics (Morflex, 1996). Examples of these citrates

include aqueous-based pharmaceutical coatings, extra corporeal tubing, blood bags, I.V. solution containers and catheters (Morflex, 1996).

Among the citrates ATBC is one of the leading alternatives to phthalates currently being investigated. Recently, in late 2010, the manufacturers of VG introduced a new formulation to the market where they have substituted BPBG with a citrate plasticiser (Dentsply, 2014).

In a comprehensive study by Dhiman (2004), a range of citrate based plasticisers were used, with increasing molecular weights, as potential replacements for the traditional phthalate plasticisers. Mechanical properties, water absorption, solubilities and gelation characteristics were studied compared to three commercial materials namely VG, CC and GC. From the results Dhiman recommended high molecular weight citrate plasticisers as good substitutes for conventional phthalates used in commercial tissue conditioners.

2.5.2.3 Biocompatibility of Plasticisers

The biocompatibility of phthalates is questionable as from different studies it has been shown that they have toxic effects; they may be cytotoxic (Okita and Hensten-Pettersen, 1991), or in some cases, like butyl benzyl phthalate, they may be carcinogenic (Ellis *et al.*, 1979). In addition, the European Union Regulation 793/93 (2006) has also limited the use of certain phthalate plasticisers including BPBG because of its toxic effects.

According to a study by Okita and Hensten-Pettersen (1991), all four phthalate containing commercial tissue conditioners studied, namely VG, CC, KF and GC, showed cytotoxicity. However CC and KF were more cytotoxic than GC and VG. Mouse fibroblasts were used to check the cytotoxicity up to 15 days according to the ISO technical report 7405-1984. CC contains BB (87%) and DBP (4.5%), KF contains DBP (85%), GC contains BPBG (80.9%) and DBP (8.9%) and VG contains BPBG (94.6%) as listed in Table 2.1 (page 36) and Table 2.5 (page 50).

Some phthalate plasticisers such as DBP and BBP have shown estrogenic activity in the human body. These plasticisers have an effect on estrogen receptors due to their chemical structure, especially the phenolic rings (Jobling *et al.*, 1995). An *in vitro* study conducted by Hashimoto *et al.* (2003), on four commercial tissue conditioners, namely CC, TC, HC and Denture Soft, tested estrogenic activity using E-screen test and MCF-7 estrogenic tests. The number of cells was assessed by measuring total protein content using sulforhodamine B assay. Cell proliferation indicated estrogenic activity. Results showed estrogenic activity in all four commercial materials containing phthalate plasticisers, as shown in Table 2.1 (page 36) and Table 2.5 (page 50).

Tay *et al.* (2012) studied the effect of water storage and heat treatment on cytotoxicity of different denture liners including a tissue conditioner (Dentusoft) containing DBP. The cytotoxicity was measured by immersion of specimens in distilled water at 37°C for 24 and 48 hours. A quantitative method was used to measure the number of viable cells of mouse fibroblasts. The study concluded that Dentusoft alternated between slight cytotoxic to non-cytotoxic materials with 75%

cell viability in average over 48 hours. Storage in water and heat treatment had no effect on the cytotoxicity.

Cytocompatibility of the citrate plasticisers is well established in the literature (Morflex, 1996). In general the citrates have an image of being non-toxic due to the fact that citric acid is a naturally occurring product in citrus fruits and also as carbohydrates of human metabolites (Wilson, 1995). Among the citrates ATBC is a prime candidate currently being investigated as substitute for phthalates.

Nishijima *et al.* (2002) conducted a study to examine the estrogenic activity of ATBC compared with conventional phthalates using three estrogenicity assays (i.e. yeast two hybrid system assay, competition binding assay and E-screen assay), and cytotoxicity on human gingival fibroblast and living skin equivalent. They looked at the acute toxicity, mutagenicity, carcinogenicity and reproductive toxicity, and found that ATBC was more cytocompatible than the other plasticisers used in commercial tissue conditioners and it also had no estrogenic activity. Estrogenicity is related to the phenolic compounds (presence of benzene ring) (Jobling *et al.*, 1995; Hashimoto and Nakamura, 2000). Phthalate plasticisers, such as BPBG, have a benzene ring in their structure as shown in Figure 2.3 and citrate plasticisers are aliphatic as shown in Figure 2.4 hence they show no estrogenic effect. The authors also suggested ATBC as a candidate for replacement of phthalates plasticisers in tissue conditioners (Nishijima *et al.*, 2002), since they found no estrogenic effect with it.

Meyers *et al.* (1964) studied the toxicity of TEC, ATEC, TBC, and ATBC in rats, mice, frogs, and rabbits for 2 weeks. Histopathological examinations revealed no damage to the liver, kidney, lungs, and spinal cord in the animals. Johnson Jr (2002) assessed the safety of ATEC, ATBC, ATEC and acetyl trioctyl citrate in cosmetic products. He reviewed different studies on acute, short-term, sub-chronic and chronic oral toxicity, neurotoxicity, cytotoxicity, genotoxicity and carcinotoxicity of these materials and concluded that they were safe for use. Similarly Hirata-Koizumi *et al.* (2011) focused on the effects of 6 different plasticisers, including ATBC and TBC, on the oral exposure, and ATBC was found to be least toxic among all plasticisers.

Thus it can be said that citrate plasticisers, especially ATBC, are safe to use as an alternative to the phthalate plasticisers.

2.5.3 Ethanol

The physical properties of a tissue conditioner do not only depend on the choice of the plasticiser used, but also on the amount of ethanol used. Ethanol, a highly polar molecule, is a necessary additive as it rapidly swells the polymer powder and facilitates its dissolution in the plasticiser. The amount of ethanol required to produce a clinically acceptable gelation time depends upon the particle size and molecular weight of the polymer (Braden, 1970a). Takamata *et al.* (2007) showed that ethanol content in various commercial tissue conditioners varied from 13.9% to 0.4%.

The ethanol can also influence water absorption where a high ethanol content will result in higher absorption (Braden and Causton, 1971; Jones *et al.*, 1988). A high ethanol content will also result in greater weight loss and shrinkage of the material because the plasticiser leaches more readily, thus leading to more rapid hardening of the material, in water or in the oral environment (Harrison, 1981). Furthermore, the leached ethanol can irritate the oral mucosa, whereas some patients find the taste and sensation of the ethanol objectionable. Also use of ethanol (alcohol) is also forbidden in many religions like Islam (Quran 5: 90-91). Hence Braden (1970b) considered reducing the ethanol content in the material; however lowering the ethanol content increased the gelation time (Murata *et al.*, 1994; Murata *et al.*, 2001a). The effect of ethanol on the properties of tissue conditioners is further discussed in section 2.6.

There had been some concerns about ethanol as a risk factor for carcinogenicity and in February 2007, the WHO's International Agency for Research on Cancers classified that ethanol in alcoholic beverages was carcinogenic to humans. However, ethanol containing products such as mouthwashes and dental products are safe to use when intended for a short period of time (Braden, 1968; Lachenmeier, 2008; Lachenmeier *et al.*, 2009). Tissue conditioners are only used as temporary denture lining materials, up to 3 months in the mouth (Graham *et al.*, 1991b) and as there is complete leaching of ethanol in the first 24 hours (Jones *et al.*, 1988), they are considered to be safe to use (Nishijima *et al.*, 2002).

2.6 Properties of Tissue Conditioners

2.6.1 Gelation

The gelation of tissue conditioners is important in determining the handling characteristics of the material indicating how much time the dentist has to apply it to the denture and to adapt to the oral contours.

The gelation of tissue conditioners has previously been characterized by using a reciprocating rheometer (Jones *et al.*, 1986) and oscillating rheometer (Murata *et al.*, 1993; Murata *et al.*, 1998b; Li, 2007; Hassan, 2007). Gelation is rheologically described by three stages i.e. pre-gelation (sol), sol-gel transition and post-gelation (gel) (Murata *et al.*, 2005). Gelation begins when the powder is mixed with the liquid. Initially ethanol swells the polymer beads allowing penetration of the plasticiser. The result is polymer chain entanglement and the formation of a physical gel (Parker and Braden, 1990).

There are many factors that affect the gelation time. These include the amount of ethanol, molecular weight of the polymer powder, ball milling the polymer powder, the class of the plasticiser, powder to liquid ratio and temperature. These are discussed subsequently.

Ethanol content plays an important part in gelation process. Increasing the amount of ethanol decreases the gelation time. In a study conducted by Parker and Braden (1990) it was shown that by using 60/40 BMA/EMA copolymer powder with BPBG

the gelation time was decreased from 63 min to 31.5 min when the ethanol content was increased from 2% to 4%. Similarly, in 2001, the same authors showed that the gelation time of VG was decreased from 16 min to 6 min when the ethanol amount in the liquid containing BPBG was increased from 4% to 8% (Parker and Braden, 2001). The effect of ethanol on gelation time has also been reported by other authors and all are in agreement that increasing the ethanol content decreases the gelation time (Dhiman, 2004; Li, 2007; Parker and Braden, 1996; Jones *et al.*, 1986).

Braden (1970a) showed that a smaller particle size of polymer powder needed less ethanol than a larger particle size polymer powder in order to achieve a similar gelation rate. One simple way of altering the particle size and surface morphology was by ball milling the polymer powder. It has been shown that the gelation rate was increased by using ball milled polymer powder that produces finer and more irregular powder particles (Parker and Braden, 2001). In addition, ball milling had a larger effect on PEMA, on both size and regularity of shape of powder particles, as it has a higher T_g than the BMA/EMA copolymer. Heat will be generated during ball milling and the copolymer powder particles, having a lower T_g may have been distorted rather than be ground (Parker and Braden, 1996). Ball milling polymer powder increased the surface area thus facilitating gelation.

Using Scanning Electron Microscopy Dhiman (2004) showed that the ball milled powder had more irregular shaped particles and additional sites of agglomeration. Irregular particles have higher surface area compared to the surface area of spherical particles. The 4 hours and 16 hours ball milled powders had a lower particle size diameter than the un-ball milled powder. All these modifications led to a

shorter gelation time for a tissue conditioner (Dhiman, 2004). Ball milling of the polymer powder not only increased the surface area of polymer particles but it was shown by Parker and Braden (1996) to also decrease the activation energy of the gelation process. This can be very useful in making formulations with reduced ethanol content that will gel in an acceptable time (Parker and Braden, 1996). Ball milling can reduce the gelation time from 82 min to 9.5 min when the PEMA powder was ball milled for 1 hour and 56 hours (Parker and Braden, 1990).

The molecular weight of the polymer powder also affects the gelation time. Use of a higher molecular weight polymer powder will lead to slower penetration of ethanol and plasticiser. Thus more ethanol is needed to control the appropriate gelation time for clinical use (Braden, 1970a). Murata *et al.* (1993) used different weight average molecular weight (M_w) PEMA polymer powders and mixed them with BPBG. Their results showed that the polymer powder with M_w between $9.4x10^4$ and $56.1x10^4$ provided appropriate gelation times and gel strength. It was shown that using M_w of 9.4×10^4 to 56.1×10^4 could reduce the gelation time from 1.57 min to 0.67min using 70%BPBG and 30% ethanol in 1.5 P/L ratio.

Copolymerisation of ethyl methacrylate with *n*-butyl methacrylate would change the T_g of the polymer. Parker and Braden (1996) showed a reduction in gelation time using copolymer of BMA/EMA. When 50/50, 60/40and 80/20 BMA/EMA copolymer were mixed with 4% ethanol and BPBG, the gelation times of these formulations were found to be ~58 min, ~32 min and ~4 min respectively.

Jones *et al.* (1986) reported that molecular weight or molar volume of plasticisers influenced the gelation. The formulations of tissue conditioners that contain plasticisers of higher molar volume were found to give longer gelation times. (Parker and Braden, 1990; Li, 2007). A higher powder to liquid ratio resulted in a shorter gelation time and increased gel strength (Murata *et al.*, 2001a; Li, 2007; Dhiman, 2004).

The gelation time can be affected by changing the temperature of the tissue conditioner, especially in the range of 20 – 40^{o}C. This property is important in clinical use of the tissue conditioner as the material has shorter gelation time at mouth temperature than at room temperature (Parker and Braden, 1996).

Gelation time is a critical property for the clinical use and a number of factors play a vital role in giving a clinically acceptable gelation time. Some of them are controlled by the user like powder to liquid ratio, which can eventually alter the ethanol amount. Dentists, dental assistants/nurse usually alter the recommended powder liquid ratios to suit their need (quick gelation) but don't realize that the properties of the materials are also changed. One alternate could be a pre-gelled material which can save the dentist time and the resulting material will have consistent properties.

2.6.2 Hardness and Compliance

Softness is a major factor in the clinical efficacy of the tissue conditioner. During use in the mouth the material should be soft enough not to harm the underlying soft

tissues. The softness of a material can be represented by its compliance or hardness value.

Compliance is a measurement associated with the depth of deformation. The higher the value of compliance the softer is the material. Hardness, on the other hand is the resistance to deformation. The lower the value of hardness the softer is the material.

Compliance can be measured using the formula (Graham *et al.*, 1990):

$$compliance = \frac{\Delta}{th} \times \frac{1}{stress} \qquad \textbf{Eq 2.1}$$

where Δ = depth of penetration of indentor

th = the total thickness of the specimen

stress is defined as “force per unit area” (Graham *et al.*, 1990).

The standard methods for measuring the hardness of elastomers and polymers use either Shore A and D, or the International Rubber Hardness Degree (IRHD) methods N, H, L and M; these test methods are specified in ISO 868 (ISO, 2003) and BS 903/ISO 48 (BS, 1995) respectively.

Shore hardness specifies procedures for measuring the hardness of materials by means of a durometer of two types: type A for softer materials and type D for harder materials (ISO, 2003). The method allows measurements at variable times for penetration of indentation. The hardness is inversely related to the penetration and is dependent on the modulus of elasticity and the viscoelastic properties of the

material (Mohamed and Aggag, 2003). The shape of the indenter, the force applied and the duration of test affects the outcome of the results. The Shore durometer is made up of a reference presser foot, an indenter, an indicating device and a calibrated spring that produces force on to the indenter (Basfar, 1997). The units of hardness range from 0 for full penetration of the indenter to 100 for no penetration. The Shore A hardness test is a popular test for elastomeric biomaterials, such as impression materials, soft lining materials, and tissue conditioners.

Measuring Shore A hardness of specimens is affected by the thickness of the specimens (Siddiqui *et al.*, 2010). In a study by Canay *et al.* (1999) Shore A hardness was measured and it was acknowledged that the thickness of the specimens would affect the hardness results. Yahya (2003), Ali (2010) and Siddiqui (2010) showed that increasing the thickness from 1 to 6 mm of the material decreased the hardness values, and that there was not much change in the hardness values when the thickness was increased further. This is because the depth of penetration of the indenter into the material is restricted by the hardness of the underlying material when there is insufficient thickness. However in a clinical situation 2 to 3 mm thickness of tissue conditioner was recommended by Wright (1976) for its optimal use. On the other hand Murata *et al.* (2009) suggested that tissue conditioners are optimally compliant at a thickness of 1.5 to 2 mm. When comparing different studies the thickness should be taken into account as higher values may not be true values when using smaller thickness specimens in order to simulate the clinical conditions.

Starcke and his team (1972) used Shore A durometer as part of a study to evaluate the physical properties of a tissue conditioning material to be used as a functional

impression. It was shown that the Shore A hardness of commercial materials (HC, CC, Tempo and Treatment Liners) increased gradually from 30 min to 24 hours, which is the recommended duration for functional impression materials. The Shore A hardness values of HC were 5.0 and 12.8, for CC 3.6 and 6.4, for Tempo 14.0 to 25.2 and for Treatment liners 18.4 and 49.2 at 30 min & at 24 hours respectively. In this study specimens use only 2.4 mm thick, which will not give the exact hardness value as thickness has more effect on lower Shore A hardness values and also at 30 min the gel formation might not be completed leading to lower Shore A hardness values.

Murata *et al.* (1996) studied the effect of change in compliance of different tissue conditioners namely CC, CS, GC and VG, in a number of solvents, over a period of 28 days at 37°C. The immersion solutions were distilled water (DW), 10%acetone/90%water, 20%acetone/80%water and hexane. The highest reduction in compliance (~50%) was seen in hexane compared with other solutions and the majority of the changes in compliance were seen in first week. Hence there is a need to change the material within a few days of use in mouth.

Yahaya (2003) reported the effect of storage in DW, artificial saliva (AS) and olive oil on Shore A hardness, over a period of 5 weeks, using VG and experimental tissue conditioner formulations containing 50/50 BMA/EMA copolymer with 2% ethanol and ATBC. The Shore A hardness increased the most in VG specimens immersed in olive oil followed by DW and then AS. The experimental tissue conditioner specimens showed an increase in Shore A hardness in olive oil followed by DW but Shore A hardness decreased in AS. This was because the plasticisers used, had higher solubility in olive oil compared to other immersion solutions.

Ali (2010) used two experimental tissue conditioners containing PEMA powder with 5% ethanol and BPBG, or 5% ethanol and ATBC. Shore A hardness of these formulations was studied for 84 days at 37°C when immersed in DW, AS, 25% ethanol/75% water, 3% citric acid and coconut oil. The results showed that Shore A hardness increased with time in all solutions, with the highest increase in coconut oil for both materials, whereas the lowest changes were found in AS. The increase in Shore A hardness was more rapid during the first week followed by a gradual increase. Both studies by (Yahaya, 2003) and (Ali, 2010) are in agreement that Shore A hardness is effected most by oil based immersion solutions and lowest changes were found in AS.

Shanmuganathan *et al.* (2012) studied the compliance of VG, CS and GC for 4 week *in vivo* using a penetrometer. Compliance was measured at 2 hour, 24 hours, 1 week and 4 weeks. They found significant changes in compliance during the test period for all materials. The reduction in compliance depends upon the base line value of the material. CS showed lowest change in compliance (4.783 to 1.781) whereas GC showed the greatest change (4.608 to 0.592) followed by VG (3.785 to 0.489), at 2 hours to 4 weeks. These changes were linked to the leaching out of ethanol and plasticiser from the material which were measured using HPLC *in vitro.* They concluded that the loss of compliance was due to loss of plasticiser rather than ethanol and that using a large molecular weight plasticiser will result in lower change.

The effect of addition of antifungal drugs on Shore A hardness has been reported in a number of studies. Urban *et al.* (2014) used Softone, a tissue conditioner and Truesoft, a resilient liner and added Nystatin (500000 Unit, 1000000 Unit),

Miconazole (125mg, 250mg), Ketoconazole (100mg, 200mg), CHD (5%, 10%) and Itaconazole (100mg, 200mg) respectively, as antifungal agents. The Shore A hardness of these formulations was measured at 24 hours, 1 week and 2 weeks when immersed in DW at 37°C. The hardness of both materials increased with time and when the amount of incorporated drugs was increased.

Bertolini *et al.* (2014) incorporated 0.5%, 1% and 2% CHD in two commercial materials namely CS and Truesoft. The Shore A hardness was measured over 7 days when immersed in water at 37°C. The Shore A hardness increased with time in both materials. The increase in amount incorporated did not have an effect on CS where the Shore A hardness of the formulations with different CHD percentages was ~10 and, after 7 days, the increase was ~20. Truesoft had an increase in Shore A hardness from ~7 in 0%CHD to ~12.5 in 2% CHD and after 7 days all formulations had hardness of about 22. The authors attributed the difference in Shore A hardness between the two materials to the difference in polymer powders used in them but failed to explain the effect of CHD

In an *in vivo* study by Graham *et al.* (1990) compliance of CC, a tissue conditioner and Veltec, a soft lining material was measured. Ten patients with complete dentures were included in this study. Denture liners were applied and measurements were taken at 1 hour, 24 hours, 48 hours, 7 days 14, days and 30 days. There was a significant reduction in the compliance of the two materials at 24 hours from 1 hour measurements, and there was no significant difference between the two materials for up to 14 days of clinical use. The compliance of CC was measured on day 30 only and no explanation was given why the measurement for Veltec was not taken on day 30.

Hardness measurements can be used to calculate Young's modulus values. The indentation produced in the case of elastomers is elastic in nature. Depending upon the type of test, the indentation is either flat-ended for Shore or spherical for ISO hardness testing (Meththananda *et al.*, 2009). So the appropriate equation for the relation between force (*F*), young's modulus (*E*), radius of indenter (*a*), depth of indentation (*w*) and Poisson's ratio (*v*), for a flat-ended indenter is (Timoshenko and Gudi, 1951):

$$F = \frac{2aEw}{1-v^2} \qquad \textbf{Eq 2.2}$$

Eq 2.2, and the following equations derived from it are for a semi-infinite solid. In practice it means the dimensions of an actual test piece need to be sufficient that the stress field produced by the indentation has decayed to zero at the bounding surfaces.

Since elastomers are incompressible (Love, 1927), *v*= 0.5, and Eq. 2.2 becomes:

$$F = \frac{8}{3aEw} \qquad \textbf{Eq 2.3}$$

In the case of a spherical indenter the equation will be (Braden, 1968):

$$F = \frac{4Ea^{1/2}w^{3/2}}{3\left(1-v^2\right)} \qquad \textbf{Eq 2.4}$$

for a rubber like material v=0.5 (Treloar, 1958), so Eq 2.4 becomes

$$F = \frac{9}{16Eaw^{3/2}} \qquad \textbf{Eq 2.5}$$

Eqs 2.2 and 2.4 were originally derived by Hertz (1881). In these two cases the depth of indentation (w) against a constant force (F) is a direct function of E; there is

a direct relationship between Shore or ISO hardness and Young's modulus (Meththananda *et al.*, 2009).

Gent (1958) investigated the relationship between Shore hardness and Young's Modulus in detail and derived equation 2.6:

$$E(MPa) = \frac{0.0981(56 + 7.66s)}{0.137505(254 - 2.54s)} \qquad \textbf{Eq 2.6}$$

where s = Shore hardness

Ideally, the hardness scale should convert a modulus range of 0 to ∞ into a hardness scale of 0-100. Eq 2.6 is valid for s=100 but not for s=0, and there are small deviations at s values under 40, given in Gent's paper, based on BS 903 (1950) (Meththananda *et al.*, 2009). However Eq 2.7 does meet the criteria using error functions to generate a hardness scale as it is more accurate at lower levels of hardness.

Apparent Young's Modulus can be calculated using the following equation based on BS 903 (1950) (Meththananda *et al.*, 2009; Siddiqui *et al.*, 2010) :

$$H = 100erf\left(kE^{1/2}\right) = 100erfz \qquad \textbf{Eq 2.7}$$

Where k= 3.186x 10^{-4} $Pa^{-½}$. Now $z=kE^{½}$, hence:

$$E = \left(\frac{z}{k}\right)^2 = \frac{z \times 10^8}{3.186^2} \qquad \textbf{Eq 2.8}$$

Where z is the value form erfz statistical table corresponding to H/100.

From the values of Young's modulus compliance values can be calculated as

$$compliance = \frac{1}{Young's\ Modulus} \qquad \textbf{Eq 2.9}$$

2.6.3 Viscoelasticity

Many materials show a complicated behaviour that cannot be described as either being Hookean solids (where stress is directly proportional to strain) or Newtonian liquids (where stress is directly proportional to strain rate); these are termed viscoelastic. They can be modelled in order to determine their stress or strain interactions as linear combinations of springs (elastic component) and dashpots (viscous component). These models are used to predict the response of a material under various loading conditions and time. The elastic modulus of the spring represents the energy stored and the viscosity of dashpot as energy dissipated. The Maxwell model can be represented by a dashpot and spring connected in series. This model postulates that strain will increase linearly from t_0 (when the load is applied) to t_1 time (when load is removed) (Figure 2.5). A drawback of this model is that it does not predict creep accurately (Darvell, 2000).

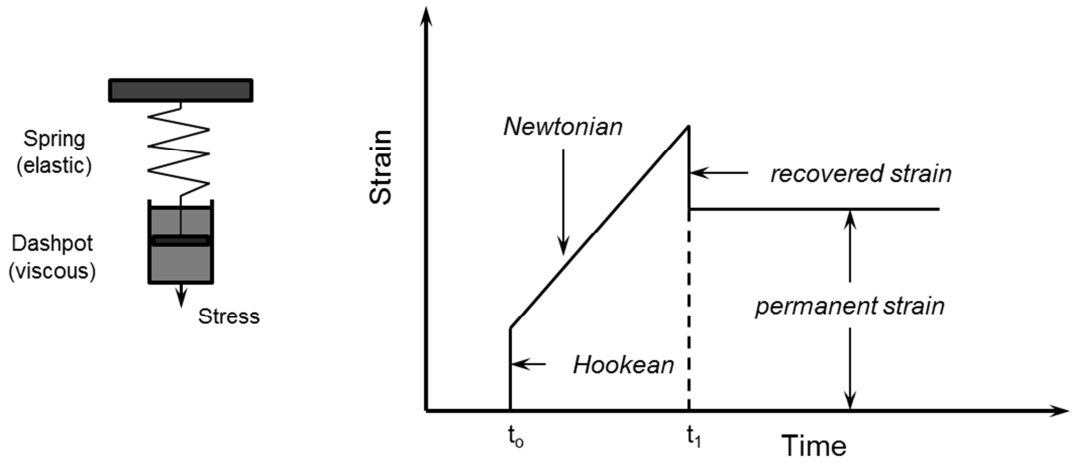

Figure 2.5: The strain response of a Maxwell model to a stress pulse (redrawn from Darvell, 2000)

The Kelvin-Voigt Model is represented by a dashpot and spring connected in parallel and it postulates that when a constant load is applied at t_0, the material deforms at a decreasing rate, asymptotically approaching the steady-state strain. When the load is released at time t_1, the material gradually relaxes to its original state. Thus this model is extremely good at modelling creep in materials but is less accurate with regards to relaxation when the stress (load) is removed (Figure 2.6) (Darvell, 2000).

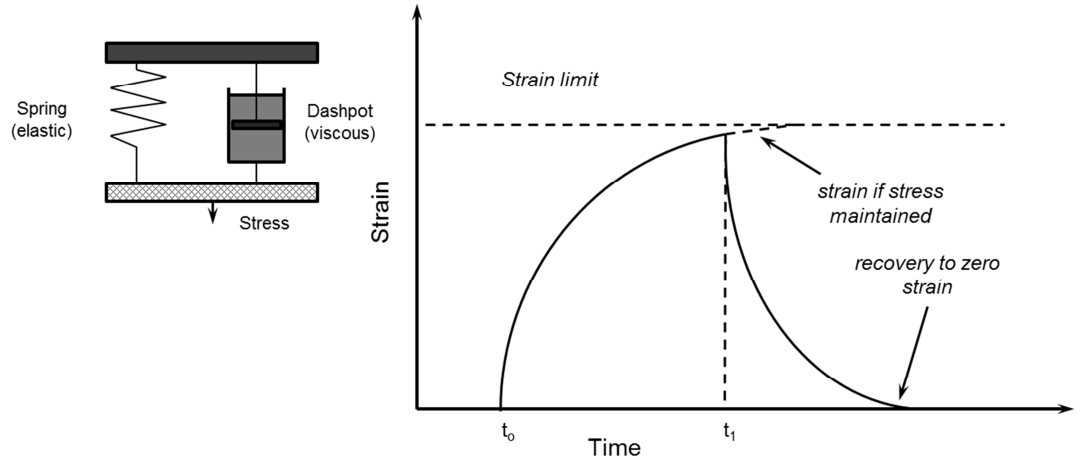

Figure 2.6: The strain response of a Kelvin-Voigt model to a stress pulse (redrawn from Darvell, 2000)

A combination of Maxwell and the Kelvin-Voigt models gives a more accurate result in predicting material responses to applied force. This behaviour, where there is a combination of an instantaneous elastic deformation, a time dependent reversible deformation, and irreversible flow, all superimposed is called viscoelastic. The plot of strain against time when a load applied at t_0 and removed at t_1 in Figure 2.7 shows all of these components present in a viscoelastic materials. When viscoelastic materials are subjected to a constant load, they experience a time-dependent increase in strain. This phenomenon is known as viscoelastic creep. As shown in Figure 2.7, when the force (load) is applied at t_0, the instantaneous elastic deformation is followed by an increase in strain with time. The Newtonian deformation continues until the load is removed at t_1. On removing this load, the elastic strain is immediately recovered, followed by an exponential recovery of the strain. The Newtonian deformation cannot be recovered resulting in permanent strain (Figure 2.7) (Darvell, 2000).

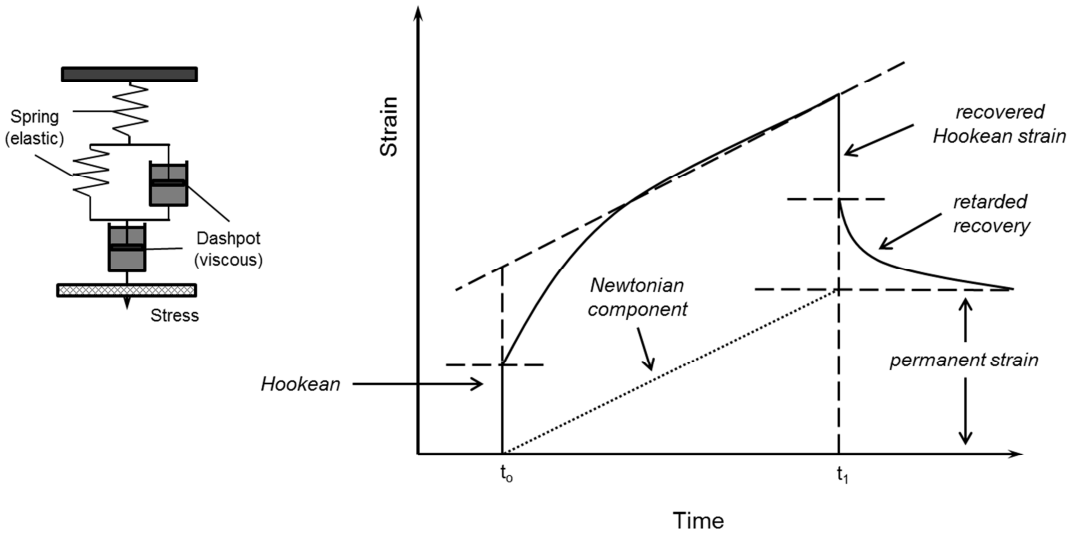

Figure 2.7: The strain response of a viscoelastic model to the stress pulse (redrawn from Darvell, 2000)

Viscoelastic behaviour of tissue conditioners is important in clinical use as it characterizes the ability of the material (see Table 2.2) to recondition abused tissues underlying ill-fitting dentures. Tissue conditioners flow viscously over a long period in response to the changes in the oral mucosa during resting, and behave elastically in response to rapid dynamic forces produced during chewing (Murata *et al.*, 2002).

Creep compliance is defined as “the change in strain as a function of time under instantaneous application of a constant stress” (Tweedie and Van Vliet, 2006). During creep test when strain is divided by stress, due to applied load, the creep compliance over time will characterize both elastic and viscous properties (Duran *et al.*, 1979).

Viscoelasticity of materials is generally measured by either applying strain and measuring the stress or by applying stress and by measuring strain (Saitoh *et al.*, 2010). Viscoelastic properties have been measured in tissue conditioners in a variety of ways such as creep test (Wilson *et al.*, 1966), stress relaxation test (Murata *et al.*, 1998a), Shore A hardness test (Dootz *et al.*, 1993) and dynamic test (Murata *et al.*, 2009).

The viscoelastic properties of a tissue conditioner are influenced by number of factors. In a study by Murata *et al.* (1994), influence of ethanol and type of plasticiser on the viscoelastic properties of different tissue conditioner formulations was investigated. In the first part of the study 0, 10, 20 and 30% by weight of ethanol with BPBG to evaluate the influence of ethanol, then BPBG, DBP and BB with 10% ethanol were also mixed with PEMA powder to evaluate the influence of type of plasticiser. Using a stress relaxation test, measurements were taken at 8 and 24 hours, 2, 4, 7, 21 and 28 days after mixing. A 10% strain was used to measure the changes in stress over a 30 min time period, where a reduction in stress indicated flow of the material. Materials with more ethanol showed more flow after gelation, but flow reduced rapidly with time of storage and with increasing the ethanol content. The formulation containing BB produced highest flow after gelation followed by DBP and then BPBG. This reflects the difference in molecular weight of the plasticisers where BB has the lowest molecular weight (212 g/mol) and BPBG the highest (336 g/mol). However the type of plasticiser used did not have any effect on the changes in viscoelastic properties with time.

Murata et al. (1998c) then studied the influence of molecular weight of polymer powder and the effect of powder liquid ratio on the viscoelastic properties using the

same technique. In this study liquids contained either BPBG or BPBG with 10% by weight ethanol. PEMA powder with average Molecular weights (M_w), which varied from 9.4 x 10^4 to 56.1 x 10^4, and the P/L ratios varied from 0.6 to 1.5 g/ml. The lower M_w powder produced the highest flow, especially after long time periods, whereas the lower P/L ratios produced greater flow, both after short and long time periods. However no effects were seen on the viscoelastic properties between either the different M_w of the polymer, or the P/L ratios. Thus the authors suggested that the viscoelastic properties can be controlled for different clinical purposes by varying the composition of the tissue conditioners (Murata *et al.*, 1998c).

Jepson *et al.* (2000) studied the influence of dietary simulation solutions on the viscoelastic behaviour of CC, VG, Coe-Soft and GC soft-liner. The study was conducted for 4 week where the specimens were immersed in DW, 8% ethanol, 50% ethanol and corn oil at 37°C. Creep compliance was measured using a pentrometer. The creep compliance reduced with time in all immersion solution however there was no consistency in pattern of reduction between the materials in different immersion solutions. The authors attributed the early reduction to the ethanol content and later changes to the loss of plasticiser.

Saitoh *et al.* (2010) studied the viscoelasticity of three commercial tissue conditioners, namely Tissue Conditioner II, TC and Tissue Care. It was concluded that all three materials had different viscoelastic properties. Tissue Conditioner II showed the highest hardness and Young's modulus followed by Tissue Care and then TC. Immersion in water showed increase in hardness and modulus of elasticity for all three materials. It was also concluded that among these three materials Tissue Care was best for use as a tissue conditioner as it showed the highest stress

relaxation, i.e. more flow, whereas the other two products were best suited as a functional impression materials. In this study a rheometer was used and equations reported by Kanie *et al.* (1992) were applied to calculate the hardness, modulus of elasticity and stress relaxation These results cannot be compared to the studies that used the standard methods for testing the hardness like Shore A or IRHD, which are the direct method of measurements.

The required viscoelastic properties are different for different applications however tissue conditioners can be used in all three clinical situations i.e. for tissue conditioning, temporary soft lining and for functional impressions. The Shore A hardness and flow (creep) properties are shown in Table 2.2 (page 44).

2.6.4 Water Absorption Characteristics

Many materials absorb water by the process of diffusion when they are placed in an aqueous environment (Braden and Wright, 1983). Similarly the constituents of the material can also leach out by the same process of diffusion. Thus it is important to determine the water uptake characteristics so that long-term durability of the material can be assessed, not only in terms of its biocompatibility, but also in terms of its functionality (Parker *et al.*, 1997b). Ideally tissue conditioners should have water uptake similar to PMMA denture base materials (~2%) (Stafford and Braden, 1968).

2.6.4.1 Diffusion Processes in Polymers

Diffusion into polymers, including the uptake of water generally obeys Fick's Laws (In, 1975).

Fick's First Law states that the flux (F) (amount of material flowing per unit area at a given point) is proportional to the concentration (C) gradient:

$$F = -\frac{D\partial C}{\partial x} \qquad \textbf{Eq 2.10}$$

where x is length of sample, and D is the diffusion coefficient. This latter property measures the speed at which the diffusing material moves through the medium and increases with temperature.

Eq 2.10 is developed mathematically to give equations for concentration at various points throughout the material at given times. Finally, it can be used to predict how much material is absorbed or desorbed as a function of time, which is highly relevant to the work in this project.

For a thin sheet of polymer the following equation applies for the earlier parts of the uptake experiment (Braden and Wright, 1983):

$$M_t = 2M_\infty \left(\frac{Dt}{\pi L^2} \right)^{1/2} \qquad \textbf{Eq 2.11}$$

M_t is the mass absorbed (or desorbed) at time t, M_∞ the final mass absorbed (or desorbed) when the process has equilibrated, and the thickness of the sample is 2L (i.e. L is half the thickness). This equation predicts that the mass change, when plotted against $t^{½}$ will be a straight line. If so, the slope (s) of the line will be:

$$s = 2M_\infty \left(\frac{D}{\pi L^2} \right)^{1/2} \qquad \textbf{Eq 2.12}$$

Hence $$D = \frac{s^2 \pi L^2}{4M_\infty^2} \qquad \textbf{Eq 2.13}$$

There are some diffusion processes that do not obey the above laws, and such behaviour is referred to as non-Fickian. The most important of these is termed Case II (Case I is Fickian diffusion), where mass change varies linearly with time. This occurs when the uptake is accompanied by relaxation of polymer chains. (Thomas and Windle, 1982).

Some cases exhibit a combination of Case I and Case II; these are generally represented by the equation (Peppas, 1984):

$$M_t = at^{1/2} + bt \qquad \textbf{Eq 2.14}$$

Polymers can absorb water by different diffusion processes and it is possible for more than one process to be involved at a time (Kalachandra and Kusy, 1991). There are number of factors that may influence the water uptake of the polymers, which are described subsequently. The presence of hydrophilic groups in the polymers which can increase water uptake because of polar attraction (Fedors,

1980). Crosslinking also limits the water uptake of polymers. This is because the material is more rigid so it will resist swelling as chains will be less mobile (Baddour *et al.*, 1965). Molecular weight of the polymer is another factor that affects the water uptake. It was shown by Turner (1987) that materials having higher molecular weight are packed less efficiently compared to the materials with lower molar weight polymers thus creating greater free volume which absorbs more water. Presence of water soluble components or impurities in the material will also lead to a higher water uptake (Fedors, 1980; Parker *et al.*, 1997a).

Muniandy and Thomas (1984) proposed that water uptake of elastomers is governed by water-soluble components within the polymer matrix that leads to a chemical potential gradient. When water molecules diffuse in (Parker and Braden, 1989) they are attracted to any hydrophilic groups or water soluble impurities present in the material (CHD and NaF if added will act as impurities) (Sample, 2001). A solution droplet starts growing around the hydrophilic group/impurity and creates an osmotic pressure gradient between the droplet and external solution which causes the material to expand or swell (Braden and Wright, 1983). The droplets continue to grow (Figure 2.8) and distort the material in the surrounding area until restrained by the material or the osmotic pressure gradient equalises (Muniandy and Thomas, 1984; Muniandy and Thomast, 1985; Braden and Wright, 1983). In tissue conditioners it is most probably the later due to their viscoelastic properties.

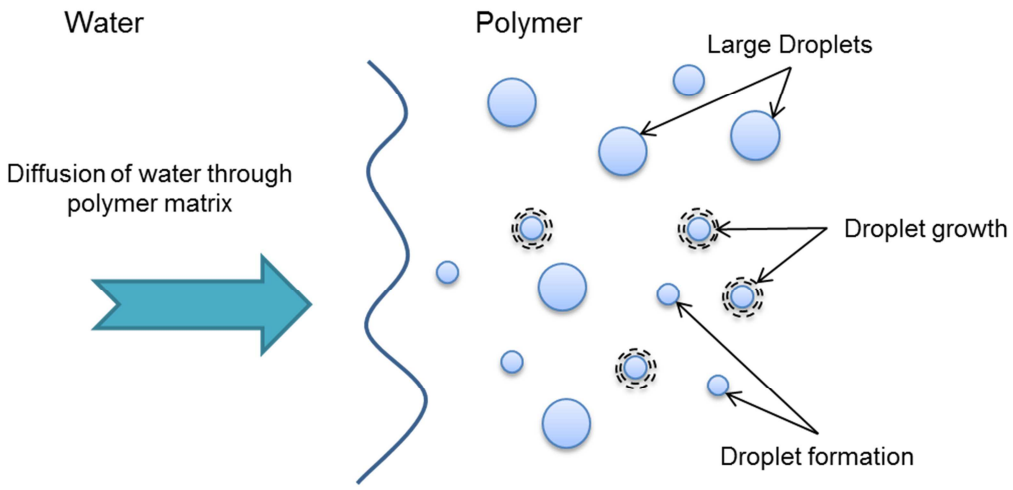

Figure 2.8: Representation of water uptake mechanism (Braden et al., 1997)

2.6.4.2 Water uptake of tissue conditioners

Water uptake study of tissue conditioners will give information on their behaviour in an aqueous environment. It also helps to study the leaching of ethanol and plasticiser from the material, thus giving a better understanding of the properties involved.

When tissue conditioners are immersed in water, water is absorbed by the polymeric phase of the gel and ethanol and plasticiser leach out into the water (Braden, 1970a; Jones *et al.*, 1988; Parker and Braden, 1990; Liao *et al.*, 2012). In a study by Braden and Causton (1971), the material with a high ethanol content showed greater and more rapid weight loss from the gel, resulting in shrinkage. This was denoted by a steady loss in weight due to ethanol diffusion when the material was immersed in water. Thus the material with a low ethanol content exhibits less change in weight when immersed in water. These latter materials are more dimensionally stable and they remain compliant for longer periods of time.

The effect of ethanol content was studied by Dhiman (2004) in DW and AS. PEMA powder was mixed with ATBC containing 2% and 5% ethanol. Results showed that increasing the ethanol increased the weight change in both immersion media. However the effect was greater in AS compared to DW. The % real uptake in DW was ~4% and 4.5% respectively, whereas in AS it was ~1% and ~1.3 respectively.

Murata *et al.* (2001b) studied the absorption, solubility and linear dimensional changes with time in DW, for a period of 21 days, of six tissue conditioner formulations including CC, VG, KF, GC, HC and SR-Ivoseal. The results showed that all materials, except SR-Ivoseal, had weight loss and shrinkage. The percentage solubility of all materials, except SR-Ivoseal, was higher than the percentage absorption. They also found that the dimensional changes were associated to the absorption and solubility, and there was a linear relationship between percentage changes in linear dimension and weight. The weight loss in the materials resulted from leaching of the ethanol and plasticiser. in SR-Ivoseal, the presence of a higher percentage of ethanol and a linear plasticiser (dibutyl sebacate) was attributed to its higher absorption and solubility.

Liao *et al.* (2012) studied the effect of immersion of Eversoft and Vertex in DW and 50% ethanol/DW solutions for a period of 52 weeks. Eversoft is a tissue conditioner containing DBP as plasticiser, whereas Vertex is a heat cured soft lining material containing ATBC as plasticiser. Results showed that Vertex and Eversoft had weight changes of ~2.7% and ~3.8% respectively in DW, whereas weight changes of ~2.9% and ~5.7% respectively were recorded in 50% ethanol/DW. The authors also reported that the uptake plots of both materials in 50% ethanol/DW showed very similar characteristics with three distinct stages. First a rapid increase in uptake

followed by weight loss and finally weight gain. The rapid weight increase was thought to be due to absorption of ethanol from the solution whereas the weight loss was indicative of plasticiser leaching along with ethanol and, the last stage is indicative of the uptake process.

Water absorption also encourages leaching of additives. Currently the main issue about the plasticisers used in dental tissue conditioners is their leachability, leading to concerns about potential toxicity (Okita and Hensten-Pettersen, 1991) and gradual hardening of these gels (Yahaya, 2003). The leaching rate of plasticisers was dependent on the composition of the tissue conditioner; the amount leached was proportional to the original content of the different plasticisers (Munksgaard, 2004). Currently there is more focus on using citrates as substitutes for the phthalate plasticisers of which ATBC is a prime candidate. This is due to the possible biocompatibility issues associated with phthalates as discussed in section 2.5.2.3 (page 52).

A commonly used method to measure the leachability of a plasticiser is by determining the weight loss of these materials, after immersing in different solutions, such as water, olive oil or artificial saliva, for a period of time (Ali, 2010; Ellis *et al.*, 1979; Braden and Wright, 1983). However, it is very difficult to determine the exact amount of plasticiser leached due to the complicated two-way exchange process between fluids and the tissue conditioner (i.e. the leaching of plasticiser and ethanol from the gel and the water uptake by the gel) (Jones *et al.*, 1988; Braden and Causton, 1971; Liao *et al.*, 2012).

The leaching behaviour of these plasticisers is assumed to be the opposite of the gelation process where they diffuse into the polymer structure when the gel forms. The data from the research of Jones *et al.* (1988) showed the amount of plasticiser leaching from soft lining materials was about 10-40 times more than the level of ester that would normally be obtained from food and environmental sources. It was also estimated that the maximum amount of phthalate ester leaching from a tissue conditioner was ~12mg/14 days, which is only one tenth of the most stringent ADI specified value for safety. It was also shown in the same study that the plasticisers with a low molecular weight, such as benzyl salicylate and benzyl benzoate, presented with higher leaching and that the leaching of phthalate esters was facilitated by ethanol (Jones *et al.*, 1988).

The leaching behaviour of a plasticiser has been shown by Graham et al. (1991b) to be higher *in vivo* than *in vitro* (immersed in DW) tests for two commercial materials, which used poly (ethyl methacrylate) polymer powder, gelled with ethanol and a phthalate ester. In this study, Coe-Comfort lost 31.1±12.4% mg/g average plasticiser *in vivo* compared to 13.41±1.11 mg/g *in vitro*, in 14 days. Veltec lost 11.8±3.3% mg/g average plasticiser *in vivo* compared to 8.47±0.73 mg/g in vitro at 30 days (Graham *et al.*, 1991b). The *in vitro* tests were carried out in distilled water whereas *in vivo* the material would be exposed to saliva and various food stuffs. *In vitro* measurements, by Ali (2010), in food simulating fluids have shown plasticisers are more readily leached in fatty food substitutes such as coconut oil when compared to artificial saliva (AS), 3% citric acid and 25% ethanol/water mix.

Effect of additives on water uptake

Tissue conditioners are widely studied as vehicles for delivery of anti-fungal drugs (Thomas and Nutt, 1978; Parker *et al.*, 1997a; Chow *et al.*, 1999; Radnai *et al.*, 2009; Urban *et al.*, 2014) so the effect of incorporation of these drugs on the water uptake is also important. As explained in section 2.6.4.1 (page 75) when antifungal drugs are added they act as impurities which attracts more water molecules thus leading to an increased water uptake (Fedors, 1980; Parker *et al.*, 1997a).

Parker *et al.* (1997a) studied the effect of incorporation of 0.9% and 9% chlorhexidine diacetate (CHD) on the water uptake of two tissue conditioner formulations for a period of 4 weeks in DW at 37°C. PEMA and an 80/20 BMA/EMA copolymer were used. The PEMA powder was mixed with a solution of 90% BPBG plasticiser and 10% ethanol, while the BMA/EMA powder was mixed with BPBG only. The formulation containing PEMA powder showed water uptake of -0.49% in 4 weeks, which increased to 2.55% and 9.6% when CHD was added at 0.9% and 9% respectively. Similarly BMA/EMA formulation had a water uptake of 2.3% which increased to 3.8% and 7.4% when CHD was added at 0.9% and 9% respectively. The authors concluded that addition of water soluble additives increased the water uptake and, the higher uptake in the case of the PEMA based material, was attributed to the presence of ethanol.

Sample, in 2001, investigated ATBC as a substitute for phthalates in different tissue conditioners and as a potential drug delivery vehicle. ATBC was used with 2% ethanol and a 80/20 BMA/EMA copolymer powder with and without the addition of 0.9% & 9% CHD. He found that the ATBC containing tissue conditioner showed

lower water absorption compared to the two commercial tissue conditioners VG and CC, both containing phthalate plasticiser. The addition of CHD increased the water uptake of the material from 2.13% to 7.93% (for 0.9% CHD) and 72.39% (for 9% CHD) in 140 days of immersion in distilled water. CHD release shown in this study is much higher compared to the study reported by Parker *et al.* (1997a), however the difference between two can be attributed to the difference in ethanol content, plasticiser and time scale.

Hassan (2007) studied the use of an ATBC based tissue conditioner as a potential drug delivery system. The effect of incorporating CHD alone and in combination with sodium fluoride (NaF) on water absorption characteristics was compared to a conventional BPBG based tissue conditioner. Both formulations contained PEMA powder with 5% ethanol. Water uptake by tissue conditioners was increased with the addition of CHD, and when NaF was also added along with CHD, the water uptake increased further from ~5-7% (CHD only) to ~30-35% (CHD and NaF).

The effect of additives (CHD and NaF) on water uptake in room temperature cured rigid lining materials has also been investigated. Sawtell *et al.* (1997) added 0.9% CHD into PEM/ tetra hydrofurfuryl methacrylate (THFM) system and showed that the equilibrium % uptake increased to ~2.8% compared to the control (~1.5%). Patel *et al.* (1998) showed that when 0.5% NaF was added to PEM/THFM polymer systems the % uptake of the polymers increased to ~65% in 250 days compared to control which was ~46%. All these studies are in agreement that addition of additives increases the water uptake because of the increase in osmotic gradient between the immersion solution and the liquid within the polymer (Patel *et al.*, 1998; Anusavice *et al.*, 2006; Patel *et al.*, 2001; Sawtell *et al.*, 1997).

2.7 Controlled Drug Delivery (CDD)

The pharmaceutical industry has primarily consisted of simple, fast-acting chemical compounds that are dispensed orally (as solid pills and liquids) or as injections. However during the past three decades, time release medications that control the rate and period of drug delivery have been developed. These specifically target an area of the body for its treatment (Vogelson, 2001).

There are many shortcomings in the current methods of drug delivery e.g. partial degradation of the drug in the body before it reaches its target site thus limiting its potency and therapeutic effect. Another shortcoming is patient compliance e.g. if the prescribed course is interrupted or discontinued, resulting in reoccurrence of the disease or there might be development of resistance for that drug. Thus there is a need for research into methods of drug delivery to administer pharmaceutical therapies. The safety and efficacy of current treatments may be improved if their delivery rate, biodegradation, and site-specific targeting can be predicted, monitored, and controlled. Therefore finding ways to administer medications by delivering costly, multiple-dose, long-term therapies in an inexpensive effective method of releasing them is also needed from both a financial and a global health care perspective. Administration methods that allow patients to safely treat themselves is as important as any other health care development (Vogelson, 2001; Vilar *et al.*, 2012).

Possible advantages of drug delivery vehicles over conventional systems are the ability to deliver a drug more selectively to a precise site; easier, more accurate, less frequent dosing; reduced variability in systemic drug concentrations; absorption that

is more reliable with the site and mechanism of action; and reductions in toxic metabolites (Okita and Hensten-Pettersen, 1991).

2.7.1 Mechanism of CDD

Polymeric controlled drug delivery systems can be divided into two methods i.e. temporal control and distribution control (Uhrich *et al.*, 1999).

2.7.1.1 Temporal Control

In this method the drug is delivered over a period of time or at specific times during the treatment. This type of drug delivery is advantageous for drugs which have quick metabolism and elimination from the body as shown in Figure 2.9. The drug given by injection or orally metabolises and its concentration remains in the therapeutic window only for a short time until another dose is given. In contrast, the controlled drug release system, releases the drug such that the rate of release remains the same as the rate of drug elimination thus the concentration of the drug remains within the therapeutic concentration window and gives maximum benefits (Uhrich *et al.*, 1999; Vilar *et al.*, 2012).

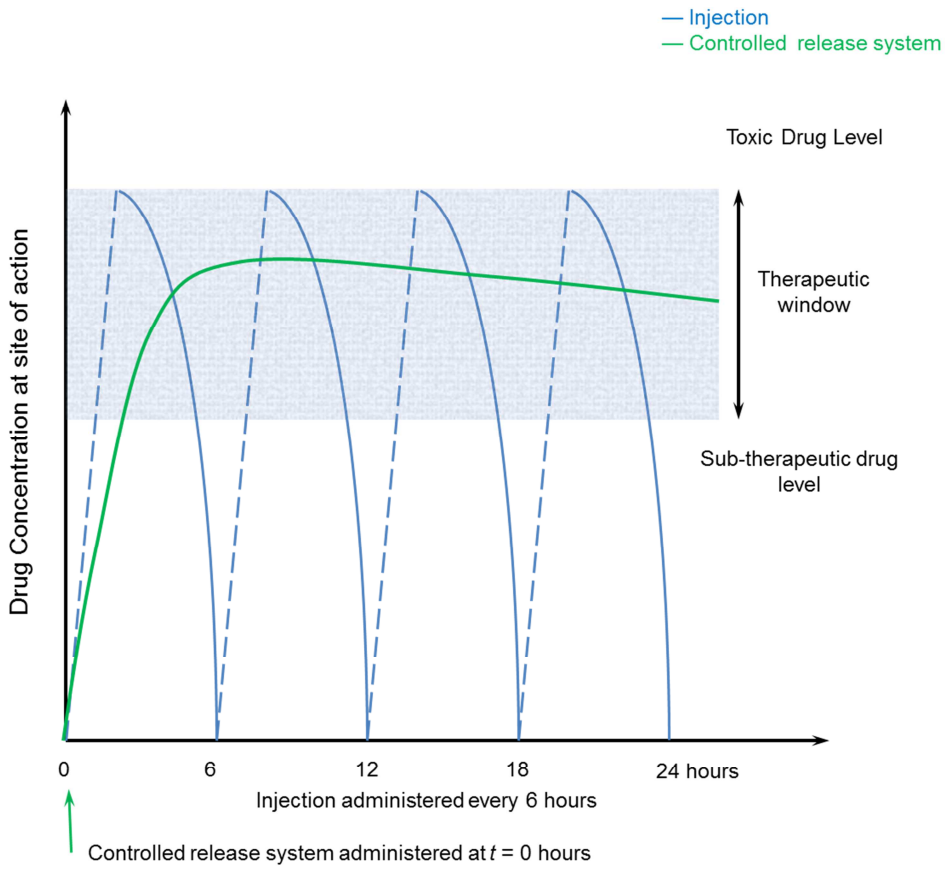

Figure 2.9 Drug concentrations in conventional injection system against temporal control release system (Uhrich *et al.*, 1999)

The polymeric matrix protects the drug molecules until they can be released from the device. This can be achieved by three mechanisms (Langer, 1990; Uhrich *et al.*, 1999):

- Diffusion controlled
- Chemically Controlled
- Drug solution flow control

2.7.1.1.1 Diffusion controlled

It is the most simple and cheapest method of controlled drug release. The drug can either be stored in a polymer sac or distributed evenly within the polymer matrix.

In the former method, a core of drug may be held in a polymer sac thus controlling release of the drug via the permeability of the polymer sac wall as shown in Figure 2.10. The release rate of the drug depends upon the thickness of the sac wall, solubility of the drug in the sac material and the concentration of the drug in the core (Langer, 1990).

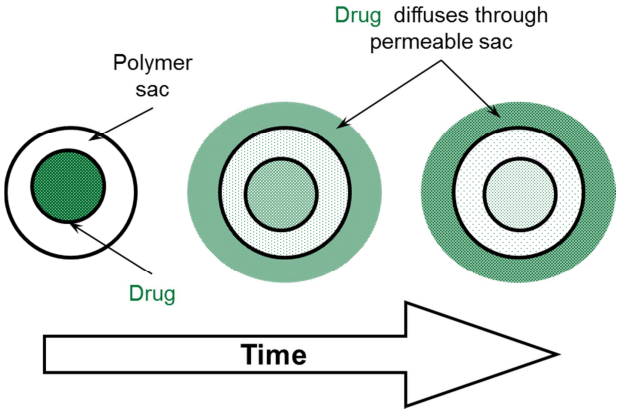

Figure 2.10: Reservoir System for Drug Delivery

In the latter method, the drug is simply incorporated into the polymer matrix from where it diffuses out through the material into the surrounding environment (Figure 2.11). The rate of release of drug is dependent on the drug solubility (in the polymer and outside the medium), its concentration and the drug diffusion coefficient (Langer, 1990).

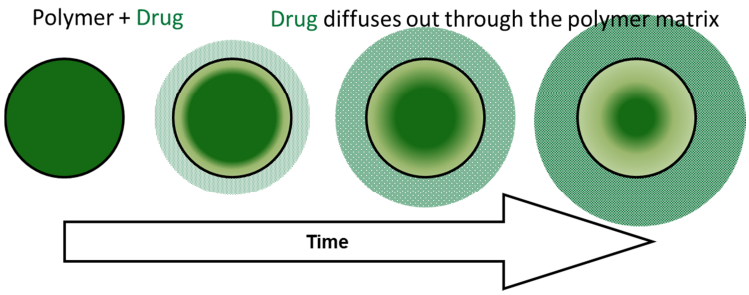

Figure 2.11: A matrix based diffusion controlled drug delivery system

2.7.1.1.2 Chemically Controlled

It is possible to design a drug delivery vehicle in which the drug is released when the vehicle encounters a specific trigger like a certain enzyme or a pH (Liechty *et al.*, 2010). One method is to bond the drug to the polymer backbone as a pendant molecule where the bond breaks by the action of water or a specific enzyme, thus releasing the drug. Another method is the use of biodegradable polymers, where the chemistry of the local environment can be used to deliver the drug. The drug is released as the polymer degrades (Figure 2.12). Several polymers, like poly lactic/glycolic acid copolymer, poly caprolactone, are used as biodegradable polymers to deliver drugs (Langer, 1990; Uhrich *et al.*, 1999; Liechty *et al.*, 2010).

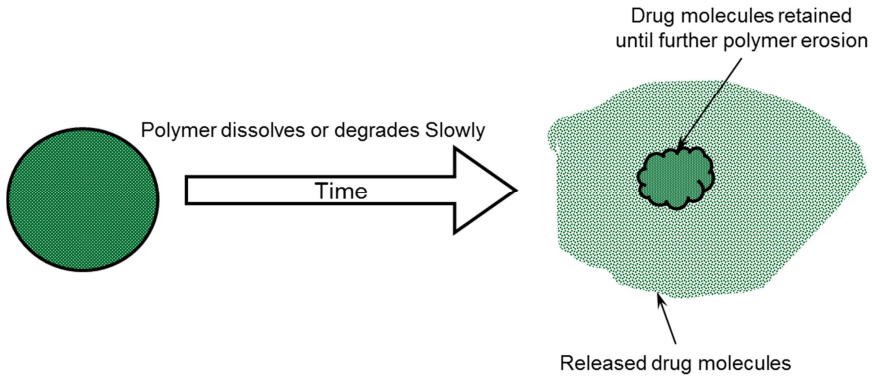

Figure 2.12: Drug release by polymer degradation (Uhrich *et al.*, 1999)

2.7.1.1.3 Drug Solution Flow Control

There are two types of mechanisms according to the type of flow control. One, where the drug is released when the polymer swells which is controlled by a solvent and the other, where drug is delivered due to the osmotic pressure gradient between the polymer and the solvent (Uhrich *et al.*, 1999).

In swelling systems, the drug is incorporated into the polymer matrix and when this polymer interacts with the environmental solvent (e.g. water based physiological fluids present in body), it swells and the polymer chains separate resulting in the release of the drug (Langer, 1990; Uhrich *et al.*, 1999).

Osmotic processes drive the later mechanism of a solvent controlled system (Figure 2.13). The water molecules cross the semipermeable membrane due to high osmotic gradient into the polymer. The drug is dissolved in the water and the pressure created by the water is relieved by the flow of the drug solution out of the polymer. The flow of the drug dissolved solution is restricted by fluid transport via

the size of the pores in the polymer thus controlling the rate of flow (Langer, 1990; Uhrich *et al.*, 1999; Vilar *et al.*, 2012).

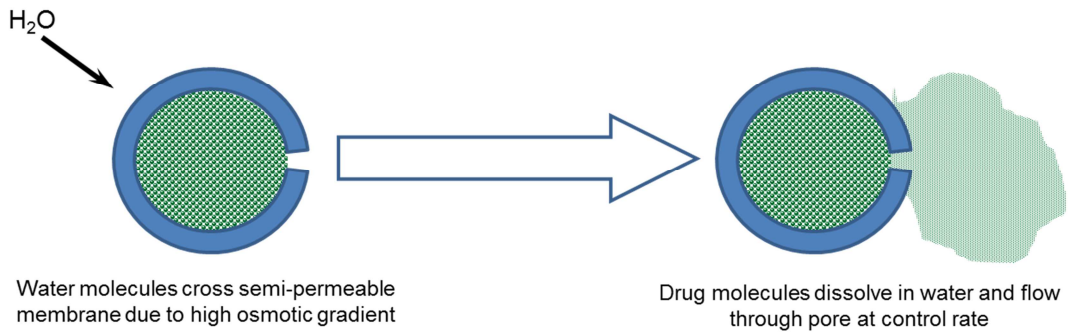

Figure 2.13: Drug molecules flow into environment through pore present in polymer (Uhrich et al., 1999)

2.7.1.2 Distribution controlled

In a distribution controlled system the drug is released where treatment is requird in the body (i.e. topical delivery). The advantage of this system is shown schematically in Figure 2.14, where a drug, when given by conventional methods like orally or by injection can only be used within the systemic window safely. To get the therapeutic effects the dose of the drug must be increased to the therapeutic window which is above the level where side effects occur so doing more harm in the body compared to the benefits. Thus by using distribution controlled system the therapeutic dose is administered at the required site of action thus the systemic side effect are avoided. Chemotherapeutic agents are a good example that can benefit from this system. Another advantage of this system is its uses for those drugs which cannot reach the site of action when delivered systemically due to natural barriers of the body e.g. a drug that cannot cross the blood brain barrier when distributed through blood, but acts on brain receptors, would be a good example (Uhrich *et al.*, 1999).

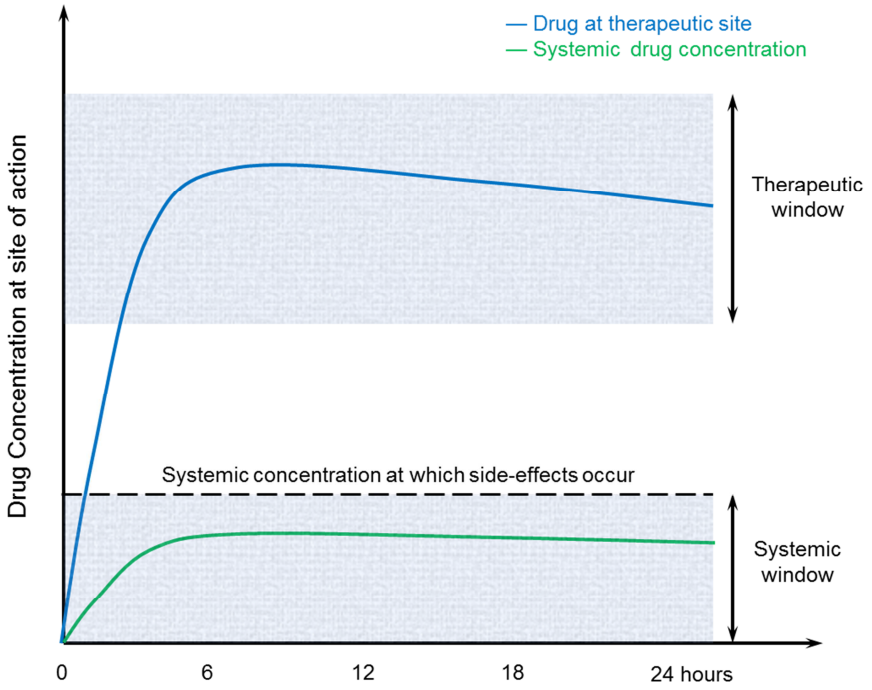

Figure 2.14 Distribution controlled drug delivery system: drug concentration level at site of action vs systemic drug concentration level (Uhrich *et al.*, 1999)

2.7.2 Tissue Conditioners as a CDD System

Tissue conditioners are ideal for controlled drug delivery systems because there is no chemical reaction during gel formation (Braden, 1970a). Any drug incorporated in the powder or liquid will remain unaffected as there will be no chance of change in the chemical structure, which may result from a chemically set material e.g. CHD decomposes when heated above 70^{o}C, so in a heat cured material where the curing of polymer is carried out above 100^{o}C the CHD will not be stable.

In this system the drug is released by a simple diffusion process as shown in Figure 2.11. Release is governed by concentration gradient in the polymer matrix and its solubility in the solvent (Sample, 2001).

Tissue conditioners were used for the first time as a CDD by Douglas and Walker (1973) when nystatin was incorporated into commercial tissue conditioners as antifungal CDD systems. Since then they have been used as potential drug delivery vehicles for the treatment of fungal infections associated with denture stomatitis (Parker *et al.*, 1997a; Eduardo *et al.*, 2001; Sample, 2001; Hassan, 2007; Geerts *et al.*, 2008; Dar-Odeh *et al.*, 2012) as further discussed in section 2.7.3.

2.7.3 CDD System in Treatment of Denture Stomatitis

As tissue conditioners are commonly used in the treatment of denture stomatitis, their matrices can be used for controlled delivery of a therapeutic agent. This idea of combining of a therapeutic agent into the tissue conditioner was first examined by Douglas and Walker (1973). According to their studies, two commercial tissue conditioners, namely Tempo and CC had fungicidal properties. When a common antifungal drug, nystatin, was incorporated in the tissue conditioner matrix, they were able to prolong the time of the fungicidal effect of the materials.

In one study by Thomas and Nutt (1978), nystatin and amphotericin B were incorporated into VG. Their results showed that VG containing amphotericin B produced a minor fungicidal effect whereas VG containing nystatin had a far greater effect. The authors concluded that tissue conditioners containing nystatin are useful for treating denture stomatitis accompanied by *Candidal* infection.

Two methacrylate-based tissue conditioner materials were developed as potential vehicles for intra- oral drug delivery (Parker *et al.*, 1997a). PEMA and 80/20

BMA/EMA copolymer were used in this study. CHD was mixed into the PEMA polymer powder to give 0.9% and 9% w/w formulations. The PEMA powder was mixed with a solution of 90% BPBG plasticiser and 10% ethanol, while the BMA/EMA powder was mixed with BPBG only. CHD release into distilled water was measured using UV/Vis spectrophotometry. PEMA containing formulation showed that 0.9% and 9% CHD formulations released 1.14mg and 10mg respectively after 4 weeks; similarly BMA/EMA containing formulations showed that 0.9% and 9% CHD materials released 4.7mg and 11.58mg respectively. Parker *et al.* (1997a) concluded that the results were encouraging for the experimental materials to be used as intra-oral drug delivery systems for additives such as CHD for the treatment of denture stomatitis.

Sample (2001) conducted a study in 2001 where he added CHD and NaF into experimental tissue conditioner formulations and found that adding NaF increased the CHD release. The CHD release in one of his experimental formulations containing 0.9% CHD with PEMA and BPBG/10% ethanol was 0.33 mg after 7 days and 1.25 mg after 140 days. The CHD release increased to 1.56 mg after 7 days and 3.84 mg after 140 days when 0.5% NaF was added into the same formulation. In the same study, Sample also experimented with ATBC as a substitute for BPBG containing tissue conditioners and as a potential drug delivery vehicle. The two different formulations containing 80/20 BMA/EMA polymer powder with BPBG or ATBC and 2% ethanol were shown to release similar levels of CHD when incorporated with 0.9% CHD. The release was 0.39 mg after 7 days and 0.7mg after 140 days for BPBG; and 0.4 mg after 7 days and 0.64 mg after 140 days for ATBC respectively.

In an *in vitro* study by Geerts *et al.* (2008) involving patients with denture stomatitis, nystatin was incorporated into VG powder by pulverization. Its effect as an antifungal denture liner was examined, comparing tissue conditioner with and without the drug in 40 patients for 14 days. Total yeast counts were performed and it was found that in the control group the total yeast count decreased for the first 4 days and then increased until the end of the test period but remained higher than the pre-treatment count. The test group showed only decreased levels till day 7 but increased after that, remaining significantly lower than the control group at the end of the test period, thus showing the advantage of adding anti-fungal drugs in tissue conditioners (Geerts *et al.*, 2008).

In a study by Radnai *et al.* (2009) CHD and miconazole gels were incorporated into VG in concentrations of 5, 10, 15 20 and 25 by volume. Sample discs were placed on Sabouraud Dextrose Agar plates which, were inoculated with *Candida albicans* prior to the experiment. CHD containing discs failed to show any antifungal activity so CHD was not investigated further. The authors suggested that either the CHD did not diffuse out of the gel or it was deactivated when mixed with VG. However VG discs containing 20% v/v miconazole were further investigated for antifungal activity over time, by immersing in water at different time periods and then placing on SDA plates. Miconazole gave a dose related antifungal effect, increasing from 10.29±1.09 mm diameter for 5% drug to 23.39±.47 diameter of inhibition zone for 25% v/v of the drug; the discs immersed in water gave an inverse relationship between immersion time and degree of inhibition (Radnai *et al.*, 2009).

In an *in vitro* study the antifungal activity of copper sulphate, borax and CHD mixed with GC was evaluated. The GC was mixed with 0.5 w/v% copper sulphate, 0.5

w/v% of equal volumes of copper sulphate and borax or 2 w/v% of CHD. The materials were placed in the wells and incubated at 37^{o}C. The mean inhibitory zone (MIZ) was measured at 24 and 72 hours. The material with copper sulphate showed maximum MIZ (26.39, 27.61) while the material with CHD showed the least MIZ (5.44, 6.33) at 24 and 72 hours (Rathore *et al.*, 2009). It should be noted that tissue conditioners are usually replaced after one week and the study does not give any antifungal activity data for that period of time.

Use of nystatin and fluconazole was investigated by Falah-Tafti *et al.* (2010) who incorporated them into tissue conditioner discs (Acrosoft) in concentrations of 1%, 3%, 5% and 10% wt/wt. All concentrations of nystatin showed complete inhibition of attachment and colonization of *Candida albicans* after 48 hours, whereas only the 10% fluconazole discs showed complete inhibition.

The release of ketoconazole and itraconazole was studied by Gupta *et al.* (2011) where two commercial materials VG and GC were used. The antifungal effect against *C albicans* was assessed after 24 hours of incubation. Inhibition of growth was significant in all drug containing materials. However itraconazole containing tissue conditioners were found to be the least effective because of the drugs interaction with the tissue conditioner. Ketaconazole in GC was found to be significantly more inhibiting than in VG, having a mean inhibition diameter of 29.45 mm and 17.95mm respectively. The authors failed to explain how the interaction between the drug and tissue conditioners affected the efficacy of the drugs.

Recently Srivatstava *et al* (2013) evaluated use of origanum oil as an antifungal agent when it is mixed with the liquid component of VG in concentrations of 10 to 65% by volume. They concluded that 65 vol% oil content was an optimum concentration for antifungal activity. They further tested this concentration to look at the effect of oil on tensile strength and surface roughness of the tissue conditioner and found that both reduced with the addition of the oil. Although these properties are important, perhaps the effect of addition of the oil on compliance and gelation time would have been more appropriate as it is more critical in the clinical use of tissue conditioners.

So far the studies in the literature are mostly focused on using different antifungal drugs as a means of treating *candidal* infections, but the effects of these additives on their properties such as compliance/creep compliance, gelation time etc. has not been widely reported in the literature. There arises a question as to whether tissue conditioners retain their physical properties and handling characteristics with the addition of antifungal drugs.

2.7.4 Anti-Fungal Agents Used in Dentistry

Some common anti-fungal drugs used in dentistry are briefly discussed in this section however main focus is on chlorhexidine's as an anti-fungal and anti-microbial use in dentistry.

2.7.4.1 Chlorhexidine

Chlorhexidine (Figure 2.15) is a bisbiguanide compound and is one of the most effective anti-plaque agent available (Walton *et al.*, 1989).

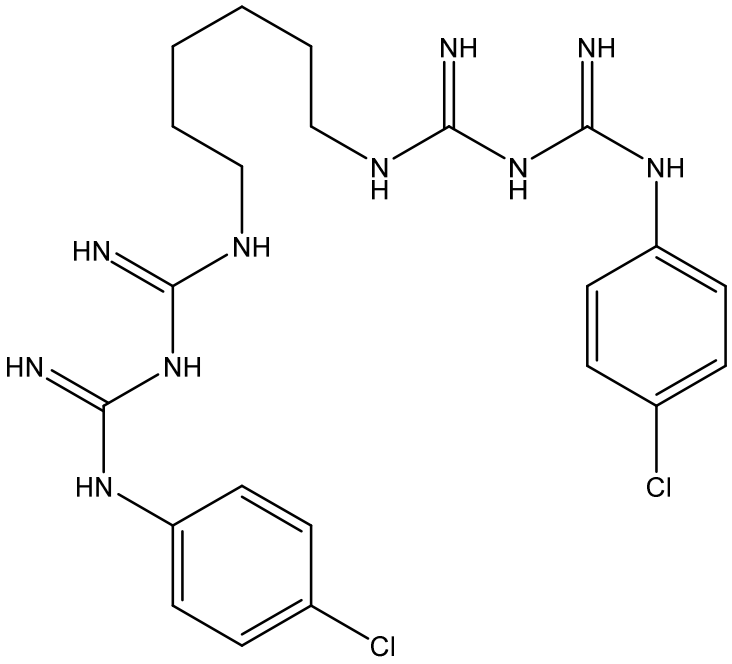

Figure 2.15 Chemical structure of chlorhexidine

It has broad antibacterial and antifungal activity and is mostly used in the form of the digluconate, diacetate and dichloride. Digluconate and diacetate are more suitable for drug delivery purposes in the oral cavity, because saliva contains chloride ions and chlorhexidine dichloride may precipitate out resulting in a reduced concentration of chlorhexidine. (Hamers *et al.*, 1996).

In vitro studies provided evidence that growth of *C albicans*, a common etiological factor in denture stomatitis, can be inhibited by CHD (Schneid, 1992; Hamers *et al.*, 1996). The mode of action of chlorhexidine as antifungal agent is not clear but it is believed to inhibit the formation of cell wall by binding to negatively charged groups in cell wall of the candida that results in intracellular material leakage and cell death

(Salim *et al.*, 2013b). The role of CHD and other antifungal agents in the treatment of denture stomatitis has been discussed in section 2.7.3 (page 92).

Chlorhexidine's use in dentistry is not solely because of its bactericidal and fungicidal properties, but also due to its retention in the oral cavity. It binds electrostatically to acidic protein groups (phosphatase, sulphates and carboxyl ions) present in the oral tissues. This increase in retention in the oral fluid means that more drug remains active for a longer period of time. In the oral environment, chlorhexidine is considered to be active above a concentration of $2x10^{-4}$mg/100ml (Rölla and Melsen, 1975).

Chlorhexidine has many applications in dentistry where, along with other antibacterial drugs they can also be used to reduce the incidence of caries (Emilson, 1994). Schaeken and De Haan (1989) studied the release of drugs from controlled release varnishes, which contained chlorhexidine and fluoride separately and a combination of both. The effectiveness of each varnish was investigated against Streptococcus mutans. The varnish containing fluoride had no effect on the microbe while varnish with chlorhexidine showed a marked reduction in the Streptococcus mutans level. There was an even greater inhibitory effect of the varnish containing both chlorhexidine and fluoride, which might suggest that fluoride may have increased the delivery of CHD. A similar effect of fluoride on release of CHD is seen in tissue conditioners as discussed in section 2.7.3 (page 92).

Guiliana *et al.* (1997) studied seven commercial mouthwashes containing cetylpyridinium chloride, chlorhexidine digluconate, hexetidine, sanguinarine, and

triclosan as active ingredients. The minimum fungicidal concentration (MFC) against six species of yeasts was determined by a broth macrodilution method and the kill-time at half the concentration of the commercial mouthwashes was also determined. MFCs were achieved with all mouthwashes, except the sanguinarine containing mouthwash. No kill-times were achieved with the sanguinarine containing mouthwash, whereas mouthwshes containing either cetylpyridinium or CHD had less than, or equal to 180 seconds, kill-time with all the species of yeasts.

There have been a few studies in the literature investigating the release of CHD from soft polymers but their release has also been investigated in rigid polymers for different dental applications. For the potential treatment of periodontal disease a study was performed by Addy and his co-workers in (1982). CHD, metronidazole and tetracycline were incorporated, into the powder component of various dental acrylics (e.g. PMMA).The release characteristics of these materials were compared. The results showed that drug release was greater when the drug concentration in the polymer was high. Furthermore, metronidazole and tetracycline leached from the material more quickly than CHD due to different properties of these drugs, but they were clinically effective in the management of chronic periodontal diseases (Addy *et al.*, 1982).

The use of CHD as an anti-fungal drug has also been studied from acrylic polymer systems besides tissue conditioners. In a recent study by Cao *et al.* (2010) discs were prepared by copolymerization of methacrylic acid and diurethane dimethacrylate. These discs were immersed in 5% miconazole or 10% CHD solutions for 24 hours. The amount of drug bound to the discs was measured using Soxhlet extraction and UV measurement techniques. The results showed 59.8±2.5

μg/cm^2 of miconazole and 45.7±2.1 μg/cm^2 of CHD bound to the discs. Their antifungal activity was then assessed using Kirby-Bauer technique at pH 5 and 7. The discs with miconazole produced a inhibition zone of 5.0±0.4 mm and CHD containing discs produced a 2.0±0.2 mm inhibitory zone showing miconazole to be more effective than CHD. The antifungal activity of the discs was sustained for up to 60 days and the drug release was faster at pH5 than at pH 7. Additionally, the bound drug was washed out with EDTA and the discs recharged with the same or a different drug. The recharge level and also the inhibition zones were found to be at a similar level to the original changed discs. This demonstrated the possible reuse of these discs.

CHD release was studied from light curable filled resins containing urethane dimethacrylate (UDMA) and triethylleneglycol dimethacrylate (TEGDMA). Leung *et al.* (2005) reported a release of ~50% CHD from a composite resin containing hydroxyethylmethacrylate (HEMA), TEGDMA, UDMA and 10w% CHD in DW for 2 weeks. Anusavice *et al.* (2006) used CHD at 9.1%, 23.1% and 33.3 wt% in a UDMA/TEGDMA filled resin and studied the release for 4 months in solutions of different pH. They showed 3.5%, 29.1% and 50.5% CHD release respectively in pH 4 compared to 1.4%, 9.8% and 11.6% release respectively at pH 6. This study showed that CHD release was increased in an acidic environment.

A study by Ryalat *et al.* (2011) used 10% w/w CHD in room temperature PMMA acrylic resin. 58.4 mg of CHD was release after 28 days. Similarly Salim *et al.* (2012) used 10% w/w CHD in poly ethyl methacrylate/tetrahydrofurfuryl methacrylate (PEM/THFM) copolymer system and measured the release for 28 days and found ~ 50% of the CHD released in DW. Salim *et al.* (2013a) also studied the

antifungal effects of fluconazole and CHD using a PEM/THFM copolymer where each sample contained 100mg of active drug. The drug release was studied for 28 days in water using UV/Vis spectrophotometer and a time kill method was used for antifungal activity against fluconazole susceptible and fluconazole resistant candida species. They found that both drugs showed antifungal properties for the first seven days, however CHD showed a more rapid (100%) kill and continued to be effective upto 28 days.

2.7.4.2 Other Anti-fungal Drugs

Anti-fungal drugs used commonly for treatment of fungal infections are nystatin, amphotericine B and imdazoles. These are usually used to treat systemic diseases (Requa-Clark, 2000). A brief description about their use in dental treatments is discussed below.

Nystatin

Nystatin is a topical antifungal drug used to treat infections such as thrush and denture stomatitis. The drug is active against yeast and fungi only but does not show any antibacterial effect. Due to the toxic effects of this drug it is not used for treating systemic disorders (Blomgren *et al.*, 1998).

Amphotericin B

Amphotericin B is effective against a number of fungi including *C albicans* but it is ineffective against bacteria. It can be used to treat systemic diseases but is mostly combined into lozenges or a suspension for use in dental applications.

Imidazoles

Imidazoles are another group of anti-fungal drugs which includes clotrimazole, miconazole, econazole, fluconazole and ketoconazole. Clotrimazole and miconazole are commonly used in dentistry. Miconazole is often used as topical application by dissolving 250 mg pill in water, it is also available in gel from which it can directly be applied on the infectious site and also to the tissue surface of the dentures. Fluconazole is another common systemic antifungal drug. It is well tolerated and has low toxicity and mild side effects but in elderly patients, who often have reduced salivary production. There is a chance of low levels reaching the oral cavity thus leading to the resistance against these drugs so limiting its use in dentistry (Samaranayake *et al.*, 2009; Siikala *et al.*, 2010).

The effect of release of some of these drugs has been studied in tissue conditioners for the treatment of denture stomatitis as discussed in section 2.7.3 (page 92) but their usefulness is limited to patients with active candida infection whereas CHD can be used not only to treat the fungal infection but also to prevent it and to improve the oral hygiene of the patients without any fear of undesirable effects of the drugs.

2.8 Aims and Objectives

Aims of this study are

- To develop an ethanol-free, citrate-based, pre-gelled system to overcome the problems associated with commercial P/L tissue conditioners.
- To investigate the release of CHD from both pre-gelled experimental and P/L commercial/experimental citrate-based tissue conditioners.
- To investigate the potential of a novel method to assess flow properties of both commercial and experimental tissue conditioners which uses Shore A hardness measurements at different dwell times.

The objectives of this study are

- To investigate the shelf life (stability on storage) of the pre-gelled tissue conditioner system over a period of 18 months using Shore A hardness measurement.
- To evaluate the physical properties of the experimental pre-gelled system and the commercial/experimental P/L formulations, which included weight changes in DW, Shore A hardness and creep compliance ratio with time when stored dry, DW and AS, and the gelation time for commercial/experimental P/L formulations.
- To evaluate the effect of addition of CHD with or without NaF on the same physical properties (described above) of both experimental pre-gelled and the commercial/experimental P/L formulations.
- To investigate the release of CHD from both pre-gelled and the P/L experimental/commercial formulations in DW.
- To study the processes involved in water uptake and CHD/F release.

CHAPTER THREE: MATERIALS AND METHODS

3 Materials and Methods

3.1 Materials

The materials used in this study are listed in Table 3.1 along with their supplier's name.

Table 3.1: List of materials used

Name	Supplier
Poly (ethyl methacrylate) (PEMA) - powder	Lucite International, UK. Batch No. B3/17180
Acetyl tributyl citrate (ATBC) - plasticiser	Vertellus Performance Materials Inc. USA. Batch No. 0000070477
n-Butyrltri-n-hexyl Citrate (BTHC) - plasticiser	Morflex Inc. USA
Viscogel (VG Old) (Old formulation)	Dentsply International, UK
Viscogel (VG) (New formulation)	Dentsply International, UK
Coe-Comfort (CC)	GC Europe
Ethanol	BDH Chemical Ltd, UK
Chlorhexidine diacetate (CHD) - powder	Sigma-Aldrich Co., UK. Batch No. 19H0417
Sodium Fluoride (NaF) - powder	Sigma-Aldrich Co., UK
Distilled water (DW)	
Artificial saliva (AS)	A S Saliva Orthana by A.S Pharma, UK

The constituents of AS (A S orthana) is listed in Table 3.2

Table 3.2: Constituents of Saliva Orthana

Each 50ml of Aqueous Solution Contains	
Component	**Amount**
Mucin Gastric	1.75g
Methylparaben	50mg
Benzalkonium Chloride	1.0mg
EDTA	25mg
Xylitol	1.0g
Peppermint Oil	2.5mg
Spearmint Oil	2.5mg
Potassium Fluoride	0.21mg
Mineral Salts	-

In this study two commercial materials Viscogel (VG) and Coe Comfort (CC) were used as commercial controls. Both materials were used in two powder/liquid (P/L) ratios; a manufacturer's recommended P/L and a higher P/L ratio of 1.8g/ml. The recommended P/L ratio for VG is 1.5g/ml, for VG Old is 1.3g/ml and for CC is 1.2 g/ml. CC was included in the study as well as VG to represent the range of commercial tissue conditioners available where VG is recommended for general application and CC more for use as a tissue conditioner.

VG was used as a commercial control in all experimental procedures. VG using P/L ratio of 1.8 was used with 1% chlorhexidine diacetate (CHD) and 9%CHD with and without 0.5% sodium fluoride (NaF).

VG containing butyl phthalyl butyl glycolate (BPBG) in liquid (VG Old) was also used to compare the effect of change of plasticiser, (the new VG contains a citrate based plasticiser but it does not state which one) and the results are presented in Appendix A2.

Two different experimental materials were used; an experimental powder liquid system (EPLS) and an experimental pre-gelled system (EPGS). The composition of EPLS consisted of 16 hours ball milled poly (ethyl methacrylate) (PEMA) powder mixed with 95% Acetyl tributyl citrate (ATBC) and 5% ethanol using a P/L ratio of 1.8g/ml. The composition of EPGS was PEMA powder mixed with ATBC in a P/L ratio of 1.2 g/ml. This ratio was finalised as appropriate after the pilot study. Both materials were also used with 1% or 9% with and without 0.5% NaF. A summary of formulations and their P/L ratios used are listed in Table 3.3.

Table 3.3: P/L ratios of different materials

Materials / Formulations	P/L ratio (g/ml)
VG Old	1.3, 1.8
VG	1.5, 1.8
CC	1.2, 1.8
VG 1% CHD, VG 9%CHD	1.8
VG 1% CHD+0.5%NaF, VG 9%CHD+0.5%NaF	1.8
EPLS, EPLS 1% CHD, EPLS 9%CHD	1.8
EPLS 1% CHD+0.5%NaF, EPLS 9%CHD+0.5%NaF	1.8
PEMA + ATBC (pre-gelled system)	1.2, 1.4, 1.6, 1.8, 2.0
PEMA + BTHC (pre-gelled system)	1.2, 2.0
PEMA + ATBC:BTHC (70:30, 50:50, 30:70) (pre-gelled system)	1.2
EPGS, EPGS 1% CHD, EPGS 9%CHD	1.2
EPGS 1% CHD+0.5%NaF, EPGS 9%CHD+0.5%NaF	1.2

3.2 Methods

3.2.1 Development of Experimental Pre-gelled System (EPGS)

For development of the pre-gelled system PEMA powder was used with two different plasticisers, namely ATBC and BTHC.

For the ATBC containing pre-gelled system, powder and liquid were mixed together using 1.2, 1.4, 1.6, 1.8 and 2.0 g/ml P/L ratios in order to select the most appropriate formulation. Shore A hardness specimens were prepared as described in section 3.2.3 and were left to gel for 16 hours at 37±2°C in an incubator wrapped in foil. After 16 hours the gelled specimens were removed from the mould. Shore A hardness and CCR were measured using the procedure described in section 3.2.4 and 3.2.6.

For the BTHC pre-gelled system 1.2 and 2.0 P/L ratios were tried using the same procedure as described above. Initially 1.2 g/ml P/L ratio was selected based on the results of the ATBC containing formulation and later 2.0 g/ml ratio was used when the formulation failed to gel in 16 hours. However the 2.0 g/ml also failed to form a coherent gel after 16 hours. To speed up gelation, 16 hours ball-milled PEMA powder was then used instead of un-milled PEMA powder. It was shown by Parker and Braden (2001) that ball-milling the PEMA powder for 16 hours decreased the gelation time from 58 min to 19 min when mixed with BPBG.

The 16 hours ball-milled PEMA powder was then mixed with BTHC using 2.0 g/ml P/L ratio only, in order to accelerate gelation, with the same procedure as described above for the ATBC pre-gelled system. After 16 hours the material again failed to form a coherent gel for hardness testing. Again using 2.0 P/L ratio the oven temperature was raised to 75±2°C, which is above the T_g of PEMA, to accelerate the gelation and to form a coherent gel. The mix again failed to form a gel so no further work was carried out with BTHC as plasticiser.

Further mixtures of ATBC and BTHC were also tried in three ratios of 70:30, 50:50 and 30:70 respectively. These liquids were mixed with PEMA powder using the same procedure as described above (section 3.2.1) for ATBC materials and the same selected P/L ratio of 1.2 was used. After 16 hours of mixing only the 30:70 ATBC-BTHC containing material failed to form a gel. Shore A hardness and CCR were measured for all the formulations that formed a gel.

The final formulation selected (based on Shore A hardness and CCR results) for further development was PEMA powder and ATBC liquid mixed in P/L ratio of 1.2 g/ml. This formulation was named as experimental pre-gelled system (EPGS).

The next stage was to evaluate the shelf life of EPGS over a period of 18 months using Shore A hardness measurements. Specimens were prepared as mentioned in section 3.2.3 and stored in an oven at 23±2°C. Shore A hardness measurements were taken at regular intervals over 18 months as shown in Table 3.4; the shore A hardness increased gradually with time so new EPGS specimens were prepared and stored in 7±2°C in refrigerator (many medical products/drugs are required to

store in a cool environment). Shore A hardness was measured at regular intervals over 18 months as shown in Table 3.4.

Table 3.4: Shelf life - time periods for hardness testing

Storage temperature	Time period for testing
23±2°C	1, 2, 4 days, 1, 5 weeks, 6, 9, 12 and 18 months
7±2°C	1, 2, 4 days, 1, 5 weeks, 4, 6, 9, 12 and 18 months

3.2.2 Preparation of Powder and Liquid

3.2.2.1 Powder

The PEMA powder was ball milled for 16 hours for EPLS formulations. A roller mill (GEC Machines LTD, Newcastle, UK) was used to ball mill the powder (Figure 3.1).

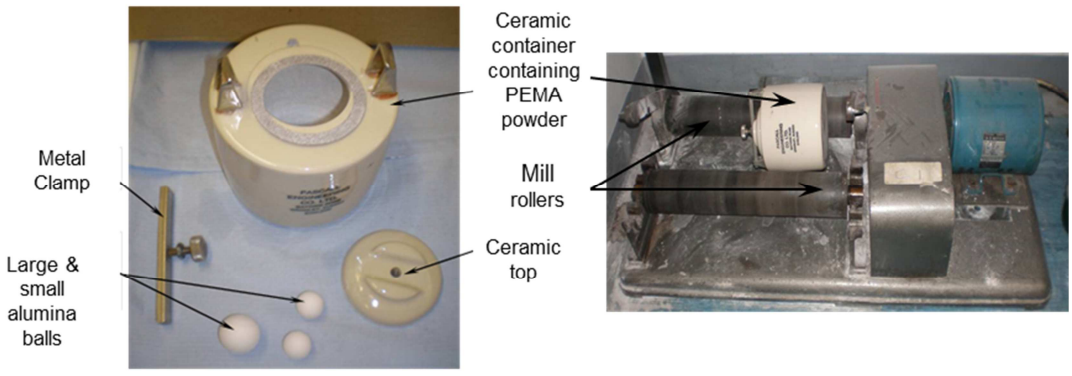

Figure 3.1: Ball milling apparatus with ball mill rollers

A ceramic container (Figure 3.1) from Pascall Engineering, Sussex, UK of 500ml capacity was used with a charge of alumina balls with two different diameters: 7 balls with an average diameter of 26.1mm and 20 small balls, with an average diameter of 18.9mm. The total weight of the balls was 500.5g. 50g of PEMA powder was placed in the jar along with the alumina balls and the jar was securely clamped by using a metal clamp on the ceramic lid and placed on the rollers of the machine for the required time (see below).

For drug delivery formulations CHD was added to the PEMA powder as 1% and 9% weight percentage with and without 0.5% NaF in EPGS, EPLS and VG. The percentage of the drug was calculated as percentage of the total powder and liquid (P/L) ratio. CHD with and without NaF was added to PEMA to give 50g of powder in total and was ball milled for 3 hours, for distributing the additives evenly. The formulations prepared with the additives used are shown in Table 3.5.

3.2.2.1.1 Particle Size Analysis

Particle size analysis is used mostly for the controlled production of powders (with appropriate particle size) used in ceramics, foodstuff and chemistry. Usually a laser technique is used to determine the particle size distribution of powders (Mishchenko *et al.*, 1999).

Theory

When an incident beam strikes a spherical particle it has a specific intensity (I_1) and wavelength. The intensity (I_s) of the scattered light is a function the scattered angle, particle size, wavelength and optical properties of the particle and the medium represented by:

$$I_1 = I_s(\theta, \lambda, d, n) \qquad \textbf{Eq 4.2}$$

Where θ is the angle of scatter, d is diameter of particle, λ is the wavelength and n is refractive index of the medium.

Two scattering theories, depending on the size range of the particles, are typically used to interpret the pattern of scattering of light and then converting it into a size distribution. These are Fraunhofer theory for particle size greater than 10μm and Mie theory for both less than and greater than 10μm particle size (Rawle, 2003).

Modern light scattering instruments use Mie theory to measure the particle size distributions assuming the particles are perfect spheres. Irregularly shaped particles are very difficult to size because of involvement of multiple parameters.

Method

A Malven Mastersizer^{TM} Type E particle size analyser (Malvern Instruments, Worcestershire, UK; Mie theory), connected to Malvern® PowerMate 286 Plus3 personal computer was used to measure the particle size of the powders of VG old, VG, PEMA and PEMA 16 hours ball milled powders.

A suspension was prepared of 2g of powder in 20ml of DW containing 6 drops of dispersant, Teepol L (Teepol Products Ltd, Surreys, UK) to keep the powder in suspension during the measurements procedure. A 100 mm focal length lens was used. The reservoir tank was filled with 900ml of DW and 3 drops of Teepol L. The laser beam intensity was checked for optimised values and a back-ground measurement was taken to set it to zero. The powder suspension solution was

added to the reservoir using a syringe (to minimize bubbles forming) with the end under the water surface until the obscuration level was 0.2 – 0.25 (recommended level), a measurement was taken. Ultrasonics were applied at a maximum level for 1 min, followed by a quick burst of stirrer to level 10, then back to level 2 and measured. This cycle of ultrasonics was repeated until D[v,0.5] which is the average particle size reached a minimum or when D[3,2] which is surface volume/mean diameter, increased indicating complete dispersion. Cumulative frequency plots of volume were obtained which were then used to calculate the mean particle diameter (D[4,3]) (Parker and Braden, 2001).

3.2.2.2 Liquid

190ml of ATBC was mixed with 10ml of ethanol to prepare a liquid of 200ml in total, with 5% ethanol and 95% ATBC concentration. This liquid was used for making specimens for all EPLS formulations as mentioned in Table 3.5

Table 3.5: Powder compositions containing CHD with and without and NaF

Formulation	Powder (50g)
VG 1%CHD	VG Powder (49.23g) + CHD (0.77g)
VG 9%CHD	VG Powder (43.1g) + CHD (6.9g)
VG 1%CHD+0.5%NaF	VG Powder (48.85g) + CHD (0.77g) + NaF (0.38g)
VG 9%CHD+0.5%NaF	VG Powder (42.72g) + CHD (6.9g) + NaF (0.38g)
EPLS	16hrs ball-milled PEMA (50g)
EPLS 1%CHD	16hrs ball-milled PEMA (49.23g) + CHD (0.77g)
EPLS 9%CHD	16hrs ball-milled PEMA (43.1g) + CHD (6.9g)
EPLS 1%CHD+0.5%NaF	16hrs ball-milled PEMA (48.85g) + CHD (0.77g) + NaF (0.38g)
EPLS 9%CHD+0.5%NaF	16hrs ball-milled PEMA (42.72g) + CHD (6.9g) + NaF (0.38g)
EPGS 1%CHD	PEMA (49.08g) + CHD (0.92g)
EPGS 9%CHD	PEMA (41.75g) + CHD (8.25g)
EPGS 1%CHD+0.5%NaF	PEMA (48.63g) + CHD (0.92g) + NaF (0.46g)
EPGS 9%CHD+0.5%NaF	PEMA (41.29g)+ CHD (8.25g) + NaF (0.46g)

3.2.3 General Sample Preparation procedure

Powder and liquid (for both P/L and pre-gelled systems) were mixed in a glass jar for 1min. The mixture was transferred to the appropriate mould (100x20x10 mm^3 polytetrafluoroethylene [PTFE] mould for Shore A hardness testing and 40x10x1 mm^3 steel mould for water absorption). The mould was placed on top of a metal plate lined with an acetate sheet. The mixture was placed in the mould which was then covered with an acetate sheet and topped with another metal plate as shown in Figure 3.2. The assembly was then wrapped in aluminium foil and clamped with

bulldog clamps and placed in an oven at 37±2°C. Before testing the mould was opened and the strips of material were taken out and any excess was removed.

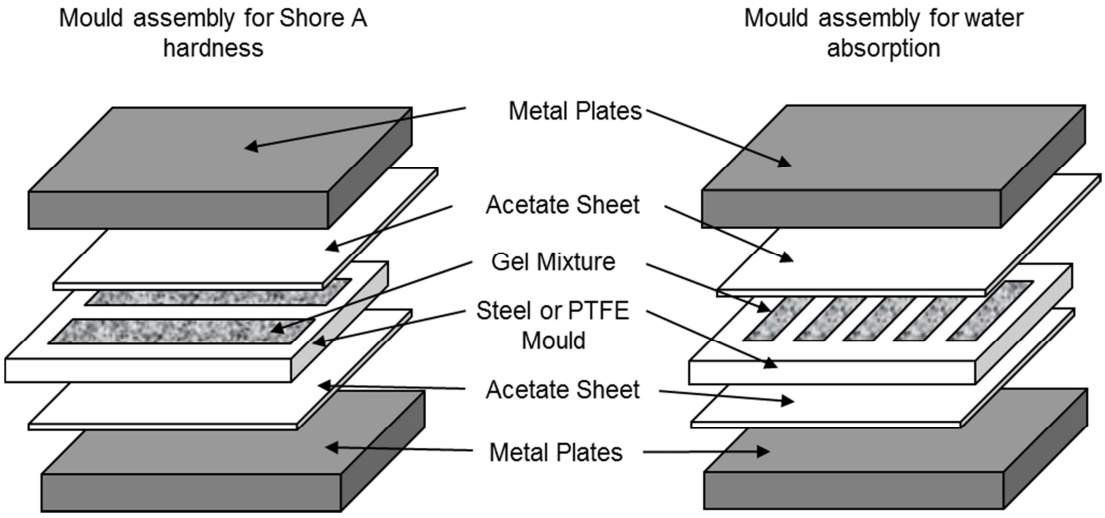

Figure 3.2: Mould assembly for specimen preparation

3.2.4 Hardness Testing

P/L ratios of all the formulations of different materials for Shore A hardness testing are shown in Table 3.3.

Specimens (100x20x10 mm^3) were prepared according to the procedure described in section 03.2.3. Shore A hardness of materials were measured after 1hr, 24hrs and 1 week after mixing for the P/L materials, whereas EPGS formulations were first tested 16 hours after mixing then after 24hrs and 1 week. After the first Shore A hardness measurements the specimens were stored either dry (always wrapped in aluminum foil until testing), in 100ml distilled water (DW) (to study the effects with

minimum factors affecting the hardness) or in 100ml artificial saliva (AS) (to mimic the oral fluids/environment), in an oven at 37±2°C for the rest of the study period.

The Shore A hardness tester (H17A, Congenix Wallace, Kingston, England) (Figure 3.3) was used to measure Shore A hardness. The indenter of the instrument is made up of a blunt-point truncated cone with indenter of 0.79mm in diameter, truncated from a cylinder of 1.1 – 1.4 mm. If the indenter totally penetrates the sample a reading of 0 is indicating a very soft material and 100 if there is no penetration thus indicating a harder material. To minimize the effect of creep, 1 sec dwell time was used to take six readings on each sample at different places, according to ASTM D 2240-05 (2010), such that the indenter was 10mm away from the edges and 10 mm apart from each reading .

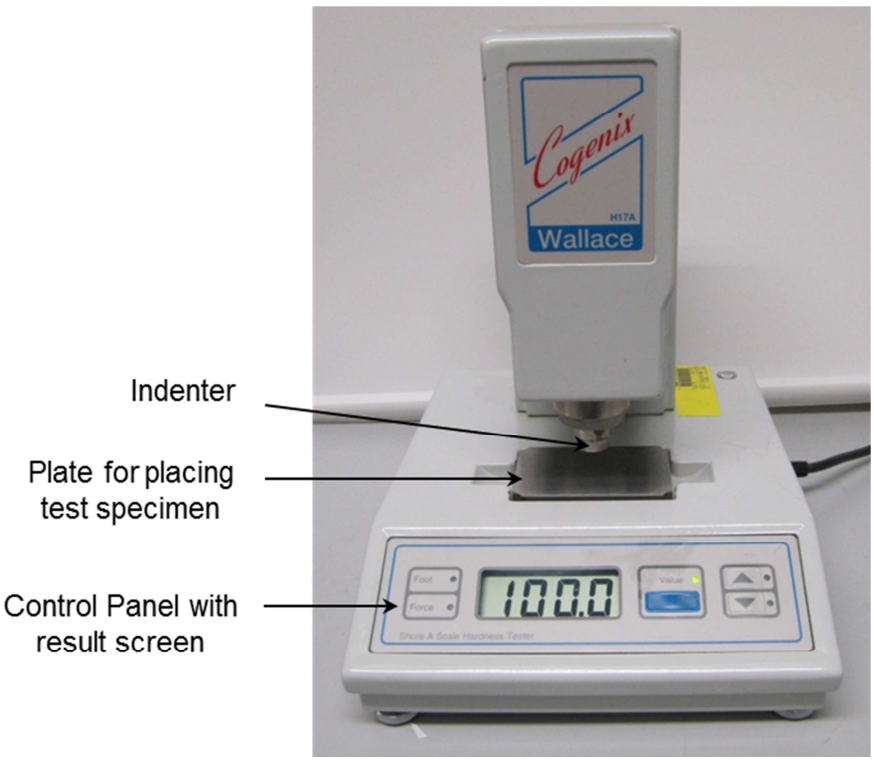

Figure 3.3: Shore A hardness tester

3.2.5 Young's Modulus

As described in section 2.6.2 (page 60) Young's Modulus can be calculated from Shore A hardness. Young's Moduli of all formulations were calculated using equation 2.8 (page 68).

3.2.6 Creep and Creep Compliance Ratio

To measure creep and creep compliance ratio (CCR), the same specimens as described in section 3.2.4 (used for Shore A hardness measurements) were used with dwell time of the indenter at 1, 5, 10, 15, 20, 25, 30 sec. Dwell time is the amount of time the indenter penetrates the specimen. Six readings 10 mm apart and 10mm away from the edges were taken according to ASTM D 2240-05 (2010).

Young's modulus and compliance were calculated using Eq 2.8 and 2.9 as discussed in section 2.6.2. From the values of compliance, CCR was calculated as

$$compliance\ ratio = \frac{compliance\ at\ time\ t}{compliance\ at\ 1\sec} \qquad \textbf{Eq 4.1}$$

Where t = dwell time.

Penetration ratio (R) was also calculated by taking the ratio of Shore A hardness at 30 and 5 sec dwell time in accordance with the ISO 10139-2.

3.2.7 Gelation Time

The gelation times of the different P/L formulations of the tissue conditioners were determined by using an oscillating rheometer (Figure 3.4). The rheometer consists of two cylindrical plates (upper and lower plate). The upper plate is removable and it remains stationary during testing with respect to the lower. The gap between the upper plate and the lower plate was approximately 1.8mm. The lower plate is connected to a rotating cam, which is attached by an iron core that moves to and fro via a wire spring allowing the lower plate to oscillate. The transducer measures this movement and is connected to a chart recorder (Linseis GmbH, 95100 Selb, Germany) which produces a trace.

The baseline trace was recorded at a chart speed of 5mm/min. A water bath (low temperature bath/circulator R series, Camlab limited, Cambridge) was used to circulate water through the upper plate to maintain the temperature, during the procedure.

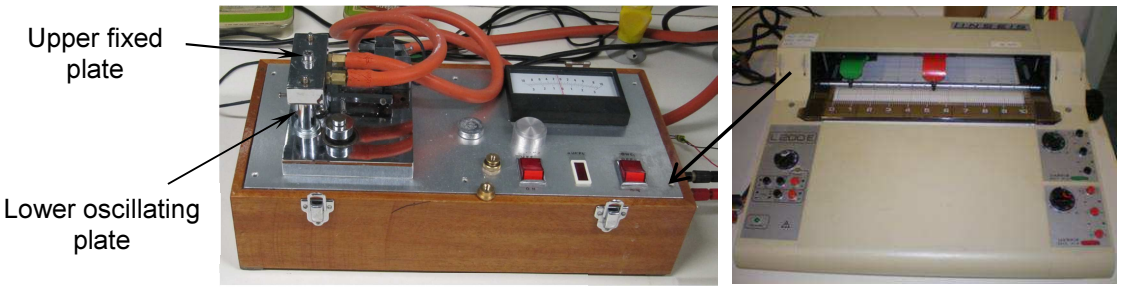

Figure 3.4: Oscillating Rheometer

The powder and liquid were mixed according to the formulations and P/L ratios shown in Table 3.3; the mixing time was approximately one minute. The mixture was transferred to the lower oscillating plate and the upper plate was then placed on top of the mixture and clamped tightly. As the material sets it resists the oscillations of the lower plate which results in a change in the amplitude of the oscillations. This was recorded on the chart recorder and a trace was produced. Gelation time was recorded from the start of mixing of material to the point when the oscillation reached a minimum value. Each formulation was repeated 5 times at 37˚± 2°C. Gelation time was taken when the rheometer trace length reached a 60% reduction (Figure 3.5) (Parker and Braden, 1996).

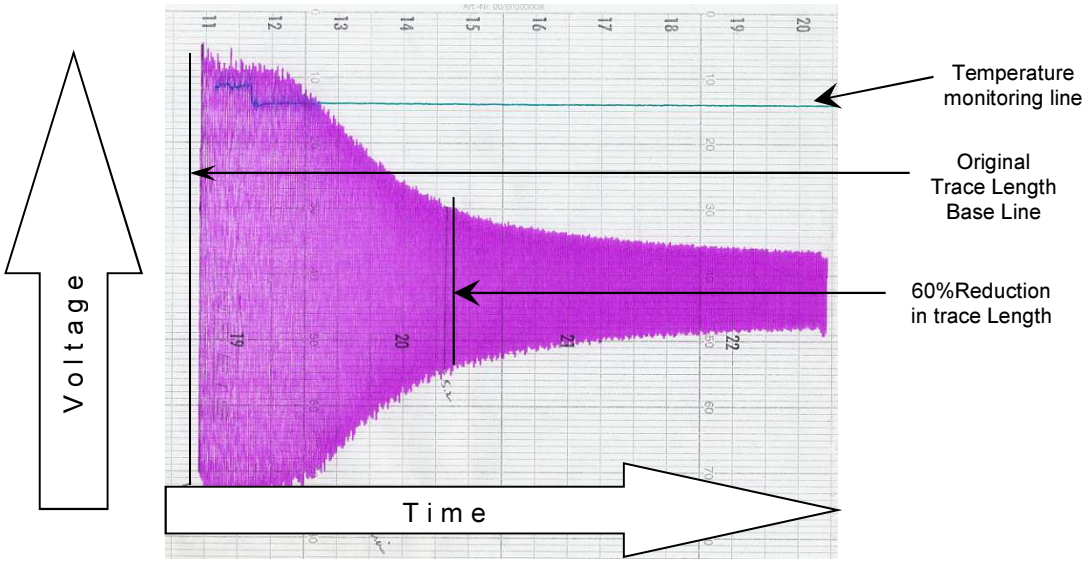

Figure 3.5: Determination of gelation time of tissue conditioner

3.2.8 Water Absorption

The powder and liquid components were mixed in the ratios shown in Table 3.3. VG old and VG were mixed according to the manufacturer's recommended ratios (1.3 for VG Old and 1.5 for VG) and 1.8 g/ml ratio. EPLS and EPGS were mixed with P/L

ratios of 1.8 and 1.2 g/ml respectively. Specimens (40x10x1 mm^3) were prepared as described in section 3.2.3.

The prepared specimens (5 specimens for each formulation) were weighed using an AE Mettler balance (Metler – Toledo Ltd, Leicester, UK) accurate to four decimal places, and then transferred to preheated glass bottles (37°C) containing 100ml of distilled water (DW). The materials containing NaF were immersed in polypropylene bottles instead of glass bottles. The bottles were stored in an incubator (Labheat Model RLCH0400, Boro Labs Ltd, Berkshire, UK) at 37±2°C. Each sample was periodically removed from the bottle, blotted dry to remove excess water, weighed and then placed back in the incubator in its' respective bottle. The specimens were weighed according to the time intervals shown in Table 3.6. The whole procedure was performed for a 12 week period because recommended time for their use in mouth as temporary lining material is 1-3 months (Graham *et al.*, 1991b).

Table 3.6: Time intervals used for the water absorption characterization

Time Period	Measurements
Week 1:Day 1	0, 5, 10, 15, 30, 60, 120, 240, 480 min
Week 1:Day 2	24 and 36 hours
Week 1: Day 3-7	48, 72, 96….336 hours (After every 24 hours)
Week 2:	After every 48 hours
Week 3-4:	Three readings a week
Week 5-9:	Two readings a week
Week 10-12	One reading a week

The water absorption for formulations containing CHD with and without NaF was carried out for 4 weeks, where the initial intervals were the same as in Table 3.6, with the exception of readings from day 3 to 14, which were taken every 24 hours, and then every 48 hours until week 4. At each time interval, starting from 60 minutes, a 5ml aliquot of immersion liquid was taken after weighing and then 5ml of fresh DW at 37°C was added so that the volume of the liquid was maintained at 100ml. These aliquots were stored in a refrigerator - Lec^{+} IST47 (Glen Dimplex Professional Appliances, UK) at 7±2°C until they were analysed using Ultraviolet/visual (UV/Vis) spectrophotometry, for detection of CHD (see section 3.2.11) and using fluoride ion spectrometry for fluoride detection (see section 3.2.12). The immersion liquid was also changed at week 1 and 2 to avoid saturation of CHD and fluoride.

3.2.9 Water Desorption

After completing the water absorption studies, the specimens were desorbed. They were removed from the respective liquids, blotted dry, weighed and stored in an incubator (Carbolite Model No. PIF120) at 37±2°C. The specimens were weighed at regular intervals using the same timing regime as described in Table 3.6 until they reached a minimum weight (equilibrium).

3.2.10 Solubility & Diffusion coefficient

Percentage weight change and solubility were calculated as a percentage of initial weight using eqs 4.3 and 4.4

$$\%\Delta W = \left(\frac{W_o - W_t}{W_o} \right) \times 100 \qquad \textbf{Eq 4.3}$$

$$\%Solubility = \left(\frac{W_o - W_1}{W_o} \right) \times 100 \qquad \textbf{Eq 4.4}$$

$$\%\, Real\ Uptake = \%\Delta W + \%Solubility \qquad \textbf{Eq 4.5}$$

where %ΔW is the percentage weight change, W_o is the initial weight of the sample before absorption, W_t is weight at time t, and W_1 is the final minimum desorbed weight.

The desorption diffusion coefficient was calculated using equation 4.6 which is appropriate for early stages of diffusion, where $M_t/M_\infty \leq 0.5$. M_t/M_∞ should be linear to $t^{1/2}$ (Patel and Braden, 1991). The equation is given by

$$D = \frac{S^2 \pi 4l^2}{16} \qquad \textbf{Eq 4.6}$$

where *D* is the diffusion coefficient, *S* is the slope of the M_t/M_∞ against $t^{1/2}$ plot and 2*l* is the thickness of the sample.

3.2.11 The Ultraviolet/Visual Spectrophotometer

Spectroscopy is a technique which measures electromagnetic radiation that interacts with molecules. A range of near ultraviolet (UV) and visible (vis) light has an energy of about 150-400 kJmol-1 in the electromagnetic spectrum which is used to promote electrons from ground state to an excited state. A spectrum is formed when light absorbed is measured as a function of its wavelength. The wavelength of

the light absorbed ranges from 150-400 nm for near UV and 400-800 nm for visible light.

Absorption spectroscopy is usually performed by dissolving a solute in a transparent solvent, where absorbance is linearly dependant on the solute concentration. So, for quantitative measurements, spectroscopic absorbance is ideal. The wavelength and strength of absorbance depends on the chemical nature and the environment of chromophores. Spectroscopy is non-destructive and very sensitive, needing only a small amount of material for analysis.

Theory

Spectrophotometers usually contain two sources of light: a deuterium lamp for UV light and tungsten halogen light for visible light region. The light passes through a monochromator and focused onto the cuvette containing the sample solution and the amount of light passing through the sample is detected by photomultiplier. In a double beam instrument a cuvette with only the solvent solution is placed in the reference beam and its absorbance is subtracted from measured absorbance of the sample. The wavelength of the beam sweeps over the UV-visible range and induces electron transition in the solvent and solute when passing through it. These transitions show characteristic peaks in the transmitted beam spectrum and can be used to measure the presence of a solute qualitatively or quantitatively. Figure 3.6 shows the schematic diagram of a double beam spectrophotometer.

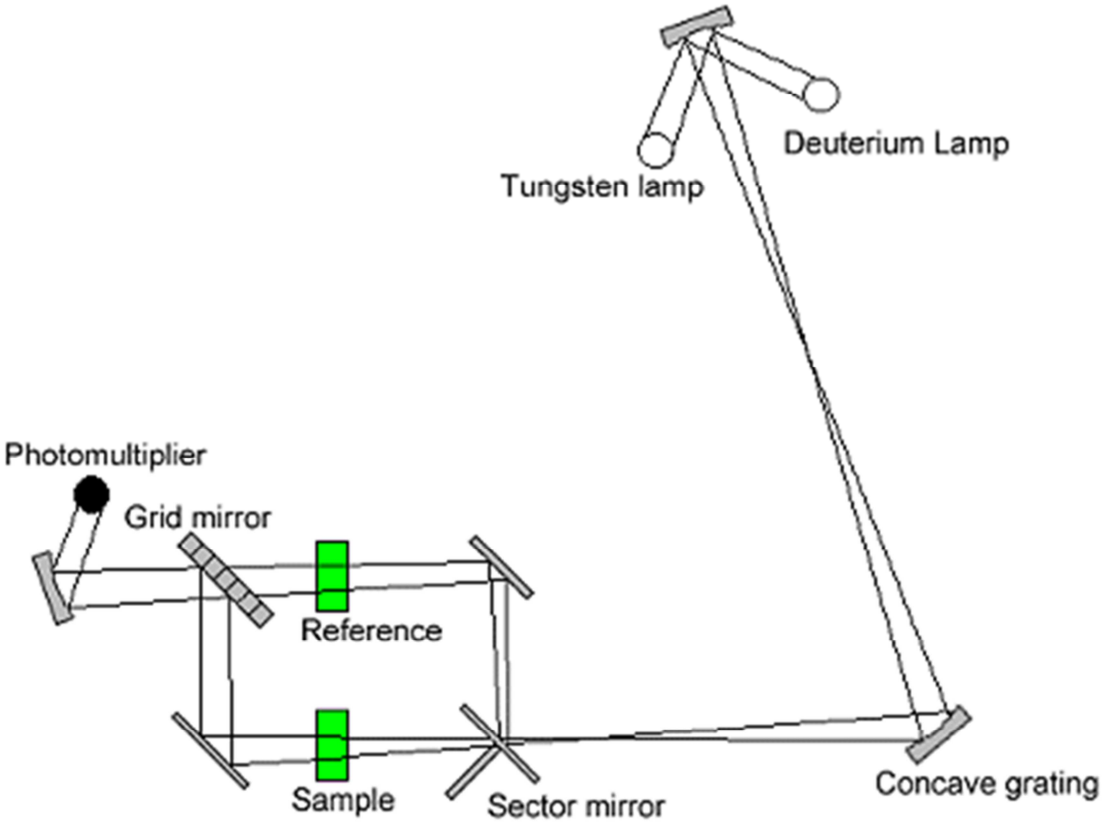

Figure 3.6: Schematic diagram of UV/Vis Spectrophotometer (UNICAM, 1995)

The absorbance (A) is related to the intensity of light before (I_o) and after (I) passage through the solution by Eq 4.7 and according to Beer-Lambert law, the absorbance depends linearly on concentration (Eq 4.8).

$$A = -log_{10}\left(\frac{I}{I_o}\right) \qquad \textbf{Eq 4.7}$$

$$A = \varepsilon c l \qquad \textbf{Eq 4.8}$$

Where in Eq 4.8 c is the molar concentration, l is the path length in cm and ε (Lmol^{-1} cm^{-1}) is the molar absorption coefficient. Thus using Eq 4.8, the absorbance can be used directly to measure the concentration of a substance in a solution.

Method

In this study UV-Vis spectrophotometry (ATI Unicam UV4, ATI Unicam, Cambridge, UK) was used to detect the amount of CHD released from drug-loaded materials into distilled water at 37°C. CHD absorbance was measured at 261nm wavelength. The instrument was set to record spectra between the wavelengths of 190 nm to 600 nm, with a bandwidth of 2nm, scan speed of 600nm/min and data interval of 1nm. The background of the instrument was set by placing fresh DW in both the reference and specimen cuvettes each time before taking the measurements. 10 calibration liquids were prepared in the concentration range of $5x10^{-3}$ g/100mls to $5x10^{-4}$ g/100mls by serial dilution of CHD in DW. Aliquots of 4ml were transferred to 5ml quartz cuvettes (AC Unicam). A calibration curve was derived, which showed the relationship of absorbance at 261nm and the concentration of CHD (Figure 4.25; page 157).

Aliquots were taken for analysis starting from 1 hour after initial immersion of specimens in water, then at each corresponding weight measurement interval, as mentioned in section 3.2.7. 5ml of solution was taken at each measurement and from this 5ml; 1ml was mixed with 9ml of DW to give a 10 times dilution. This diluted solution (4ml) was used to measure the absorbance of CHD against a DW (4ml) reference.

3.2.12 The Fluoride Ion Electrode:

For this study a fluoride ion electrode was used supplied by Orion Research Inc (USA), model 720A+. The schematic diagram is shown in Figure 3.7.

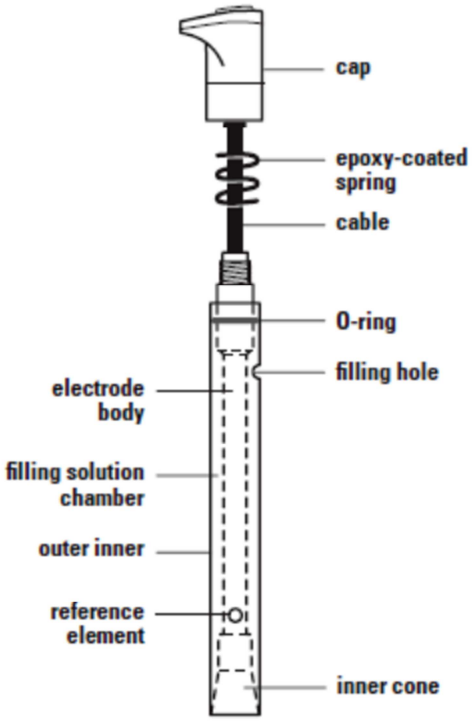

Figure 3.7: Schematic diagram of Flouride Ion Electrode (Scientific, 2007)

The electrode is made up of a sensing element attached to an epoxy body. An electrode potential builds up in the sensing element when the electrode tip is place in a solution of fluoride ions. Depending on the amount of free fluoride in the solution a potential is formed which is measured against a constant reference potential with a digital pH/mV meter or specific ion meter. The potential measurement is equal to the level of fluoride ions in the solution.

In order to get a high background ionic strength a 'total ionic strength adjuster buffer' (TISAB) is added to all fluoride calibrating standards and specimens. TISAB also works as a pH buffer and it also decomplexes any fluoride that might be bound to other polyvalent species like aluminium, iron and magnesium.

Method

The fluoride electrode used was Orion model 720A+. The tip of the electrode was washed with DW before use. The electrode was then filled with the electrode filling solution (Optimum Result A 900061, Thermo Orion). The instrument was calibrated using fluoride standards prepared in DW.

Standards in DW were prepared by serial dilution of 1000ppm aqueous sodium fluoride solution (Orion Sodium Fluoride Standard) to give concentrations of 10ppm, 5ppm, 1ppm, 0.1ppm and 0.01ppm. The meter was first set to calibration mode. 2.5ml TISAB III was mixed with 2.5ml of standard solution on the magnetic stirrer. The fluoride electrode was then immersed into the mix and the reading was recorded from the bench-top display once it reached stability. The tip was then washed with DW and shaken dry after every use. The calibration was performed in an descending order. After calibration measurement, the meter automatically calculates the slope of the linear relationship between concentration and output voltage, and then goes to the measurement mode for the test solutions to be analysed. All standards and test solutions were measured at the same magnetic stirrer speed.

Fluoride content of the aliquots taken at different time intervals as discussed in section 3.2.7 (page 118) was measured. Before fluoride measurements the aliquots were taken out of the fridge 1 hour before start of the testing to reach room temperature. 2.5 ml from the aliquot solution was mixed with 2.5ml of TISAB III solution on the magnetic stirrer. The measurements were performed using the same procedure as used for the calibration solutions.

3.2.13 Statistical Analysis

IBM SPSS Statistics version 22 software was used for the statistical analysis of the results. One way analysis of variance (ANOVA) with a post hoc Tukey's honest significant difference (HSD) test was performed to compare the means of the results. One way ANOVA compared the means of the groups to tell if there was any significant difference between the groups when p value was less than 0.05. If there were any differences among the means of the group a further post hoc Tukey's HSD was performed ($p \leq 0.05$) that indicated which group or groups were significantly different from others. Univariate analysis of variance test was also performed to check the relationship between materials and the dependent factors. If the p value is less than 0.05 then then there is a positive relationship between the dependent factors.

CHAPTER FOUR: RESULTS

4 Results

The various results obtained are summarized in this chapter. The commercial materials Coe-comfort (CC), Viscogel (VG) and Viscogel Old (VG Old) were tested using the manufacturers recommended powder to liquid (P/L) ratio and a higher 1.8 g/ml P/L ratio. Moreover, 1.8g/ml P/L ratio was used for the VG materials where chlorhexidine (CHD) was incorporated at 1% or 9% with and without the addition of 0.5% NaF. For experimental powder liquid system (EPLS) a P/L ratio of 1.8 g/ml was used throughout. The experimental pre-gelled system (EPGS) was tested using a P/L ratio of 1.2 g/ml for all formulations, which was selected following initial screening as described in section 4.1 3.2.1.

4.1 Development of Pre-Gelled System

For the development of EPGS, Shore A hardness and subsequent creep compliance ratio (CCR) were used, to select the appropriate P/L ratio and the final formulation to be used for further testing.

Figure 4.1 shows the mean Shore A hardness with standard deviations (SD) of different P/L ratios in the range 1.2 to 2.0 g/ml. Here the materials were tested 16 hours after mixing, to allow the material to form a stable gel prior to the testing. The materials containing only acetyl tributyl citrate (ATBC) showed an increase in Shore A hardness with increasing the P/L ratio from 1.2 to 2.0. The materials prepared using a liquid mix of 70/30 and 50/50 by volume, of ATBC and n-butyrltri-n-hexyl citrate (BTHC), had statistically similar Shore A hardness values, but greater than the ATBC 1.2 counterpart. The formulations containing BTHC only and 30/70

ATBC+BTHC did not gel even after 24 hours as explained in section 03.2.1 (page 108).

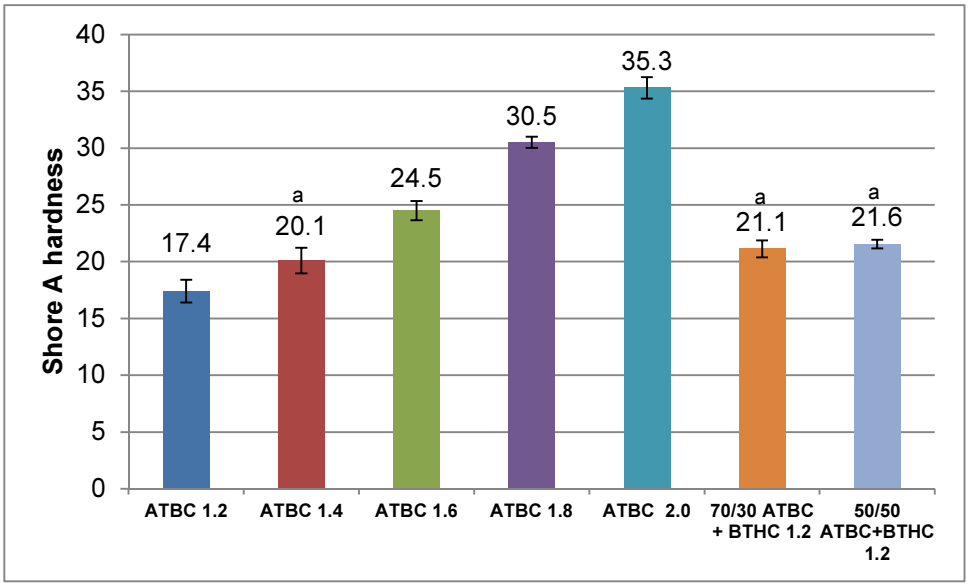

Figure 4.1: Mean (±SD; n=6) Shore A hardness of pre-gelled formulations with different P/L ratios

(No significant difference between groups with same letters; p≤0.05)

Figure 4.2 shows the comparison of the effect on Shore A hardness of increasing dwell time from 1 to 30 sec for the different formulations. With increasing dwell time the measured Shore A hardness decreased in all the formulations. Generally, the Shore A hardness values decreased by ~5 units from 1 sec to 30 sec dwell time regardless of its value at 1 sec dwell time. From the measured Shore A hardness values CCR was calculated as described in section 3.2.6 (page 117) and the results using a 30 sec dwell time are summarized in Table 4.1. ATBC 1.2 showed the highest CCR of 2.56 and ATBC 2.0 showed the lowest CCR at 1.61.

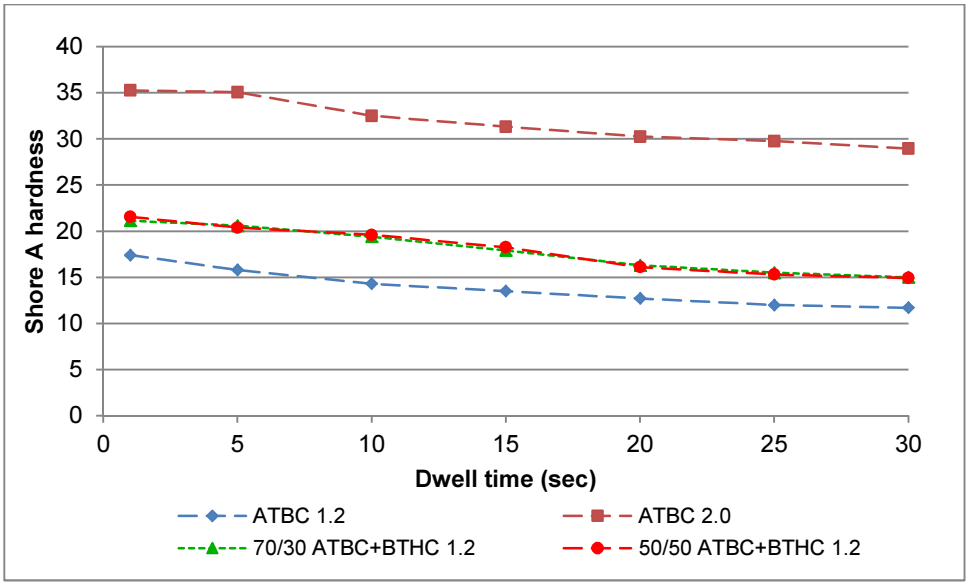

Figure 4.2: Mean (n=6) Shore A hardness of pre-gelled formulations at different dwell times stored at 37°C

Table 4.1: CCR of different pre-gelled formulations using 30 sec dwell time

Pre-gelled formulation	CCR at 30 sec dwell time
ATBC 1.2	2.56
ATBC 2.0	1.61
ATBC+BTHC 1.2	2.14
ATBC+BTHC 1.2	2.14

Based on these results, ATBC 1.2 (now referred to as EPGS) was selected for further testing as a pre-gelled system as it had the lowest compliance combined with maximum flow (CCR).

4.1.1 Shelf Life of EPGS

The selected EPGS formulation was tested for its stability over time (shelf life) by measuring the Shore A hardness over a period of 18 months at different times.

EPGS was first stored at room temperature (23°C) and the results of mean Shore A hardness with standard deviation (SD) are shown in Figure 4.3. There were 6 specimens to start with but two specimens were damaged during the experimental procedure so their data are not shown. The Shore A hardness of the material showed a constant increase from 0 day to 18 months however, this increase was more during the first 5 weeks.

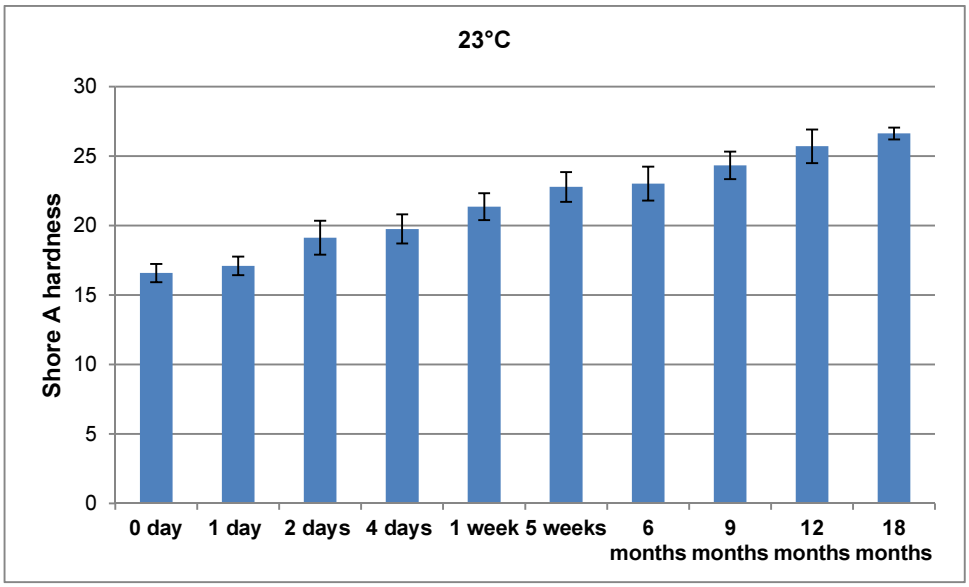

Figure 4.3: Mean (±SD; n=4) Shore A hardness of EPGS at different time periods when stored at 23°C

Figure 4.3 shows the Shore A hardness increased with time indicating that the gel was not stable at 23°C, so it was decided to evaluate the changes when stored at 7°C, as many commercial materials and drugs are commonly stored in a refrigerator. Figure 4.4 shows the changes in Shore A hardness of EPGS over 18 months when stored at 7°C. There was a significant increase in Shore A hardness from day 0 to 2. From day 2 till 18 month there were no further significant changes found in Shore A hardness at the different time periods, thus indicating a stable gel after ~2days.

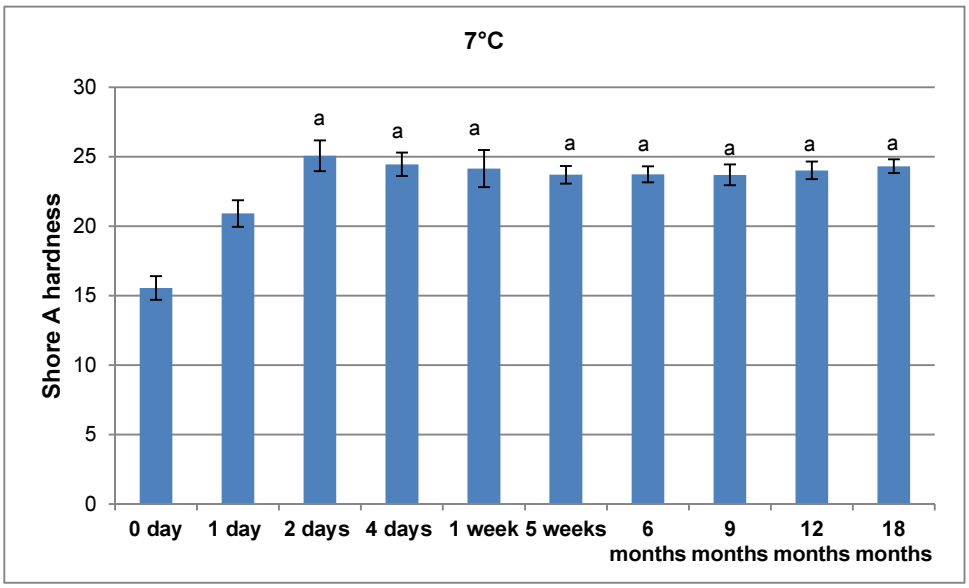

Figure 4.4: Mean (±SD; n=6) Shore A hardness of EPGS at different time periods when stored at 7°C

(No significant difference between groups with same letters; p≤0.05)

4.2 Particle Size Analysis

Particle size analysis was carried out for VG, PEMA un-milled and PEMA 16 hours ball-milled powders (used for EPLS). Particle size is one of the important factors that affect the gelation process of the tissue conditioners and also affect other properties

e.g. Shore A hardness and creep etc. Table 4.2 shows the summary of results obtained. There was a decrease in Mean particle size D[v,0.5] of PEMA after 16 hours ball-milling. D[v,0.5] of VG was smaller than PEMA powder.

Table 4.2: Mean (± SD; n=5) of Mean particle size D[v,0.5 of different polymer powders

Powder	D[v,0.5]	SD (±)
VG	32.91	0.42
PEMA	42.46	0.30
PEMA 16 hrs	40.58	0.35

4.3 Gelation Time

Gelation time was determined for only the powder liquid (P/L) formulations at 37°C because these are chair side materials and so gelation time is important. The pre-gelled system (EPGS) does not require mixing by the dentist who is only required to apply the material to the fitting surface of the denture.

Figure 4.5 shows the gelation time of VG (1.5 and 1.8), CC (1.2 and 1.8) and EPLS. The gelation time decreased with increasing the P/L ratio of CC but no significant decrease was found in VG when P/L was increased. EPLS had a gelation time longer than all except CC 1.2 where there was no significant difference.

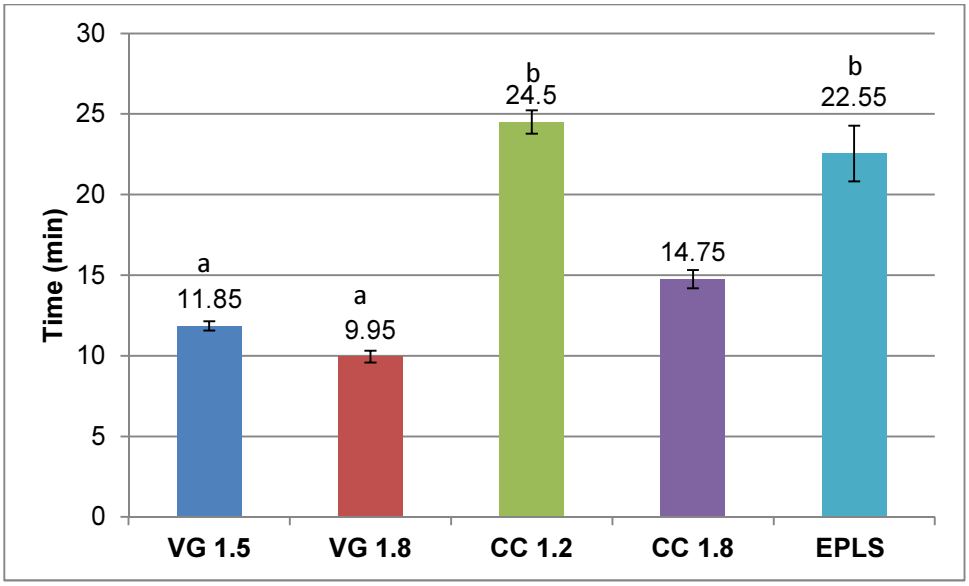

Figure 4.5: Mean gelation (± SD; n=5) time of CC, VG at different P/L ratio and EPLS at 37°C

(No significant difference between groups with same letters; p≤0.05)

VG 1.5: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.5g/ml
VG 1.8; PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
CC 1.2: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.2g/ml
CC 1.8: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml

Figure 4.6 shows the effect of gelation time on VG when CHD was added at 1% or 9% with and without 0.5% NaF. There was no significant difference in gelation time when CHD was added to VG 1.8, and when CHD was increased from 1% to 9%. Also there was no significant difference when NaF was added to 1% and 9% CHD formulations.

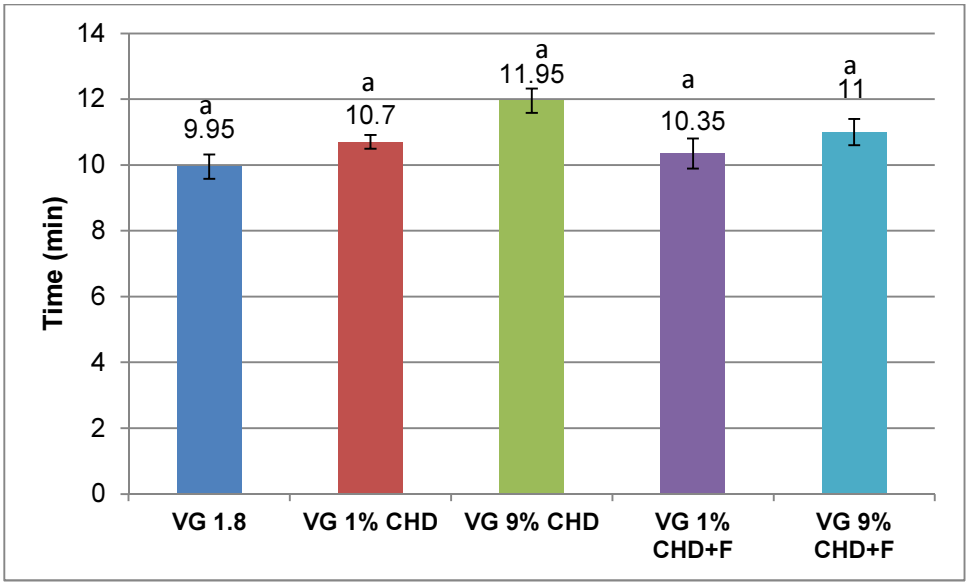

Figure 4.6: Mean gelation time (± SD; n=5) of VG with the addition of 1% or 9% CHD with and without 0.5% NaF at 37°C

(No significant difference between groups with same letters; p≤0.05)

VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml

Figure 4.7 shows the effect of gelation time on EPLS when CHD was added at 1% or 9% with and without 0.5% NaF. Gelation time increased with the addition of CHD but no significant difference was found when CHD was increased from 1% to 9% or with the addition of NaF to them.

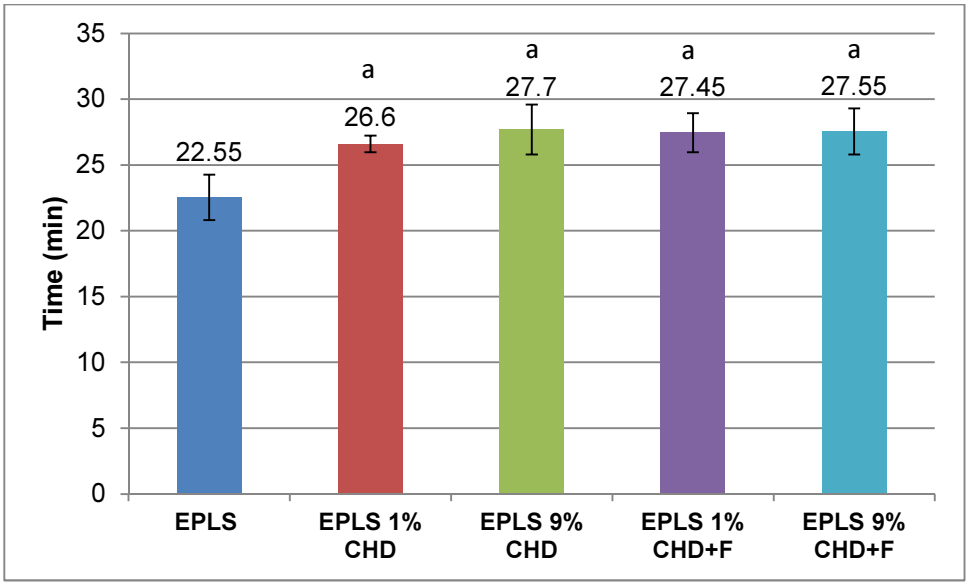

Figure 4.7: Mean gelation time (± SD; n=5) of EPLS with the addition of 1% or 9% CHD with and without 0.5% NaF at 37°C

(No significant difference between groups with same letters; p≤0.05)
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml

4.4 Water Absorption

The water absorption of different formulations of tissue conditioners was measured, as described in section 3.2.8 (page 119), for 12 weeks; the formulations with the additives were studied over a period of 4 weeks. Figure 4.8 is an example showing good reproducibility between the specimens of EPLS 1%CHD in DW at 37°C. Results are presented as plots of mean percentage (%) weight change with SD against square root of time. It should be noted that when immersed, materials not only gained weight by absorption of water but also lost weight by loss of ethanol and plasticiser etc. and the measured weight change reflects the combined effect.

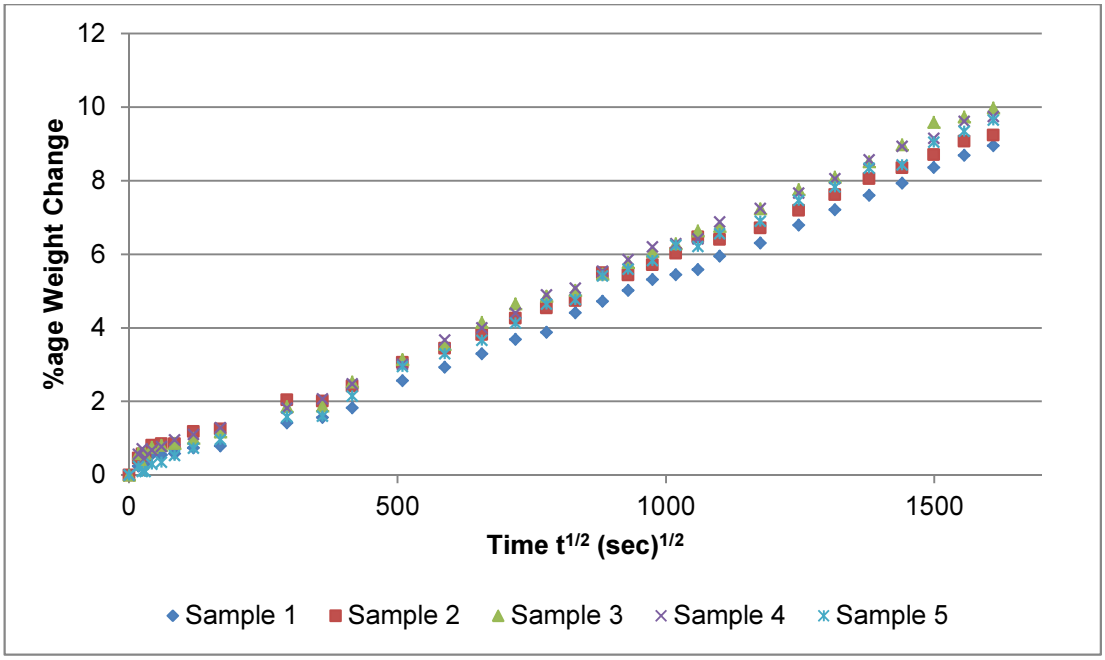

Figure 4.8: Reproducibility of water absorption plots of EPLS 1%CHD in DW at 37°C

The water uptake study of VG and VG Old was carried for 12 weeks and the results are shown in Figure 4.9. Both VG Old 1.3 and 1.8 showed similar water uptake profiles of weight loss up to day ~5 ($t^{1/2}$ =587.9) where they started to gain weight at different rates (i.e. VG Old 1.8 gained weight more rapidly than VG Old 1.3 until the end of the experimental period). VG Old 1.8 gained more weight (9.1%±0.9) compared to VG Old 1.3 (1.3%±0.2). Both VG 1.5 and 1.8 lost weight rapidly up to ~34 days ($t^{1/2}$ =1610) followed by an increase in weight to a final negative weight change of -5.8%±0.6 and -1%±0.8 respectively. This initial part is indicative of loss of material being the predominant process. VG 1.5 and 1.8 results are discussed later in detail in Figure 4.10.

The changes seen in both VG old and VG when the P/L ratio was increased was due to the decreased ethanol content in the formulation thus resulting in a lower

weight loss for the higher P/L ratios. On comparing the uptake profiles of VG Old versus VG, these reflect the roles of the different plasticisers in the two formulations (BPBG verses ATBC). This will be further discussed in section 5.4.

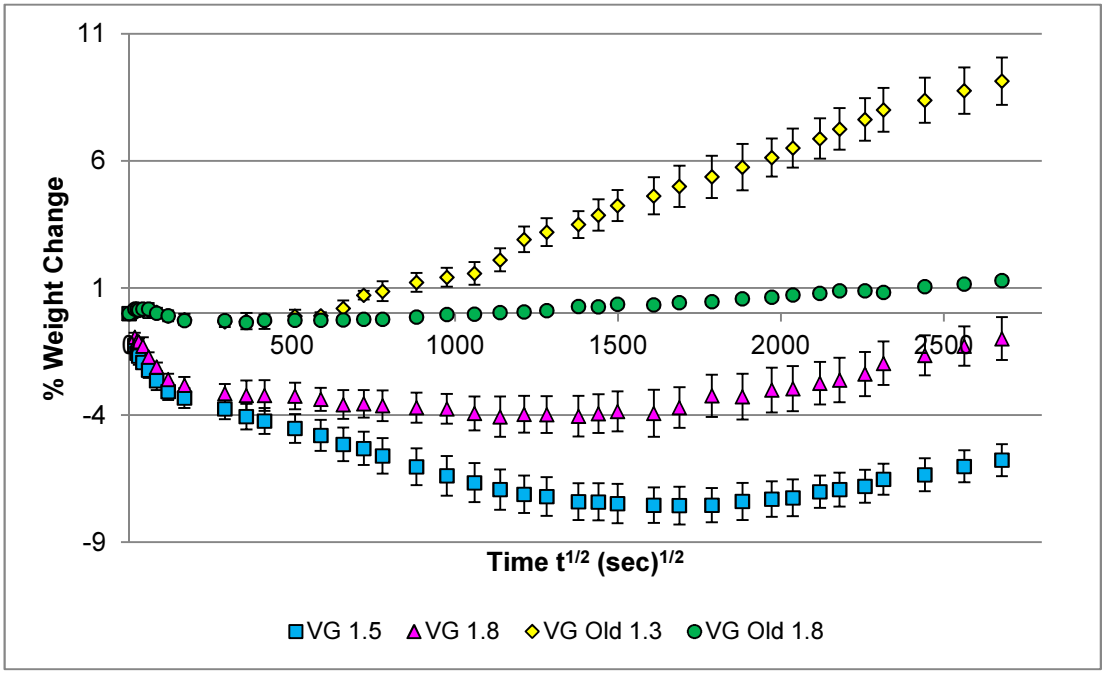

Figure 4.9: Mean (±SD; n=5) % weight change of VG and VG Old formulations in DW at 37°C

VG 1.5: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.5g/ml
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
VG Old 1.3: PEMA powder & 93%BPBG+7%ethanol; P/L ratio 1.3g/ml
VG Old 1.8: PEMA powder & 93%BPBG+7%ethanol; P/L ratio 1.8g/ml

Figure 4.10 shows the % weight change of VG at two P/L ratios i.e. 1.5 and 1.8. Both VG 1.5 and 1.8 lost weight rapidly up to ~34 days ($t^{1/2}$ =1610) followed by an increase in weight to a final weight change of -5.8%±0.6 and -1%±0.8 respectively. This initial part is indicative of loss of material. When looking at the weight change data for the first 24 hours ($t^{1/2}$ =293.9) more closely, it can be seen that the initial rapid weight loss for both VG 1.5 and 1.8 followed a similar profile up to 24 hours ($t^{1/2}$ =293.9). After this time point the weight change plots began to lose/gain weight

at different rates. VG liquid contains 6.2% w/v ethanol (see Appendix A1), so when the P/L ratio was increased from 1.5 to 1.8 the ethanol content in the formulation decreased; this resulted in a lower weight loss than VG 1.5 and highlights the role of ethanol.

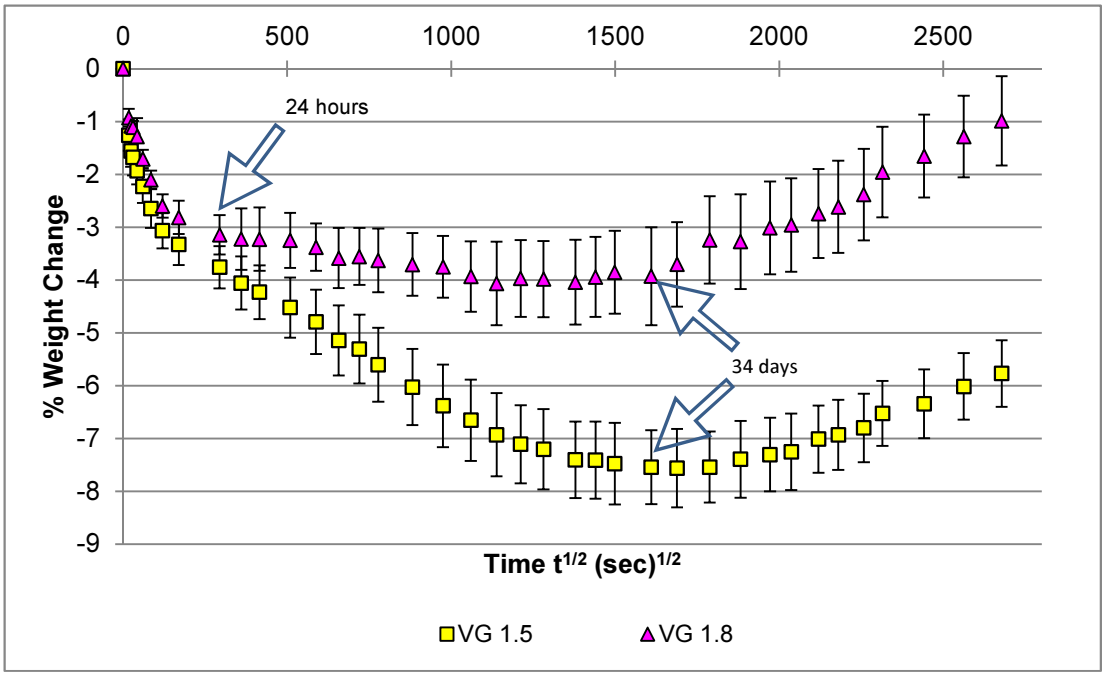

Figure 4.10: Mean (±SD; n=5) % weight change of VG at two P/L ratios: 1.5 and 1.8 in DW at 37°C for 12 weeks

VG 1.5: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.5g/ml
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml

Figure 4.11 shows the % weight change of EPLS in DW at 37°C. Note EPLS is the experimental powder liquid system with 5% ethanol compared to 10% ethanol in VG. EPLS showed a rapid weight gain in the first hour ($t^{1/2}$ =60) followed by a very small loss in weight (0.8% to 0.5%), until 8 hours ($t^{1/2}$ =169.7); this is shown more clearly in Figure 4.11 followed by a steady increase until the end of the experimental

time period of 12 weeks. Figure 4.11 clearly shows the effect of reducing ethanol in the formulation where there is a continuous increase in % weight change after 8 hours, compared to continuous weight loss as shown with the VG specimens.

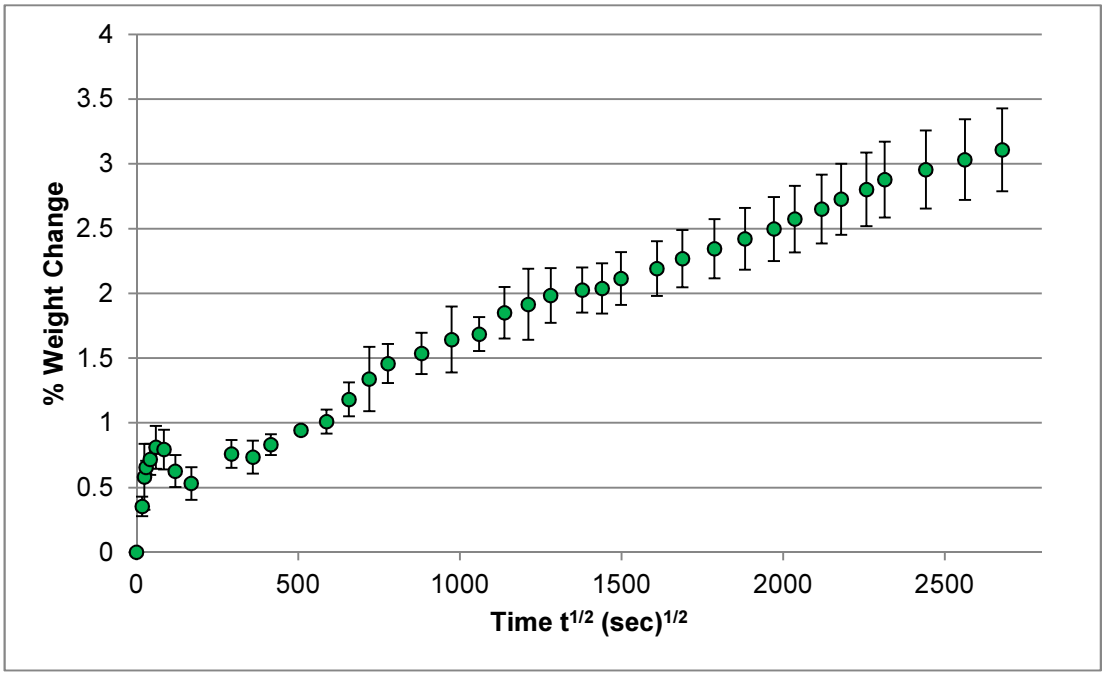

Figure 4.11: Mean (±SD; n=5) % weight change of EPLS in DW at 37°C for 12 weeks

EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml

Figure 4.12 shows the % weight change of EPGS in DW at 37°C. EPGS specimens showed a rapid weight gain in the first eight hours ($t^{1/2}$ =169.7), followed by a slower, steady increase until the end of the experimental time period of 12 weeks. Clearly the early weight losses observed with VG 1.5 and 1.8 and EPLS were not evident with EPGS. This could be attributed to the formulation containing no ethanol and this will be discussed further in the next chapter.

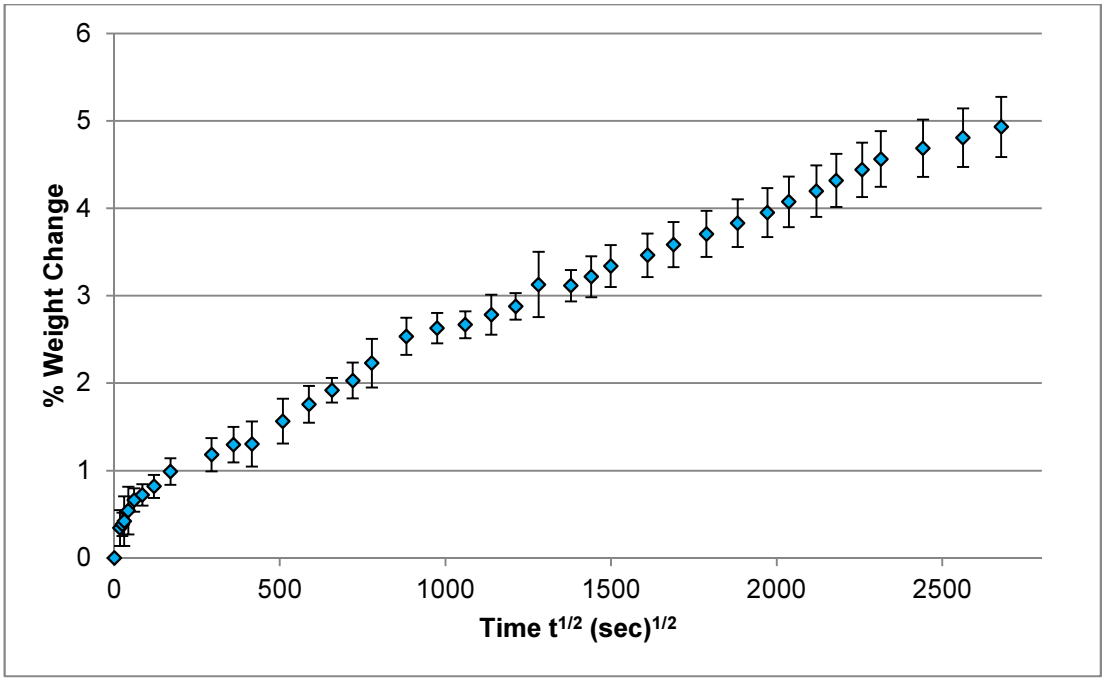

Figure 4.12: Mean (±SD; n=5) % weight change of EPGS in DW at 37°C for 12 weeks

EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

Figure 4.13 shows the % weight change for VG, EPLS and EPGS. Here the effect of ethanol in tissue conditioner formulations is seen more clearly. Materials containing more ethanol (VG 1.5 and 1.8; see appendix A1 for ethanol content in VG) show weight loss. This weight loss is due to loss of ethanol and plasticiser from the material. Reducing ethanol in the experimental formulation (EPLS) resulted in weight gain which was further enhanced in the absence of ethanol (EPGS). Since these are polymer liquid gels, the initial weight change is driven by the ethanol (where present) and subsequently ethanol and citrate leaching out. Also note that the standard deviations were much smaller for experimental formulations compared to VG 1.5 and 1.8.

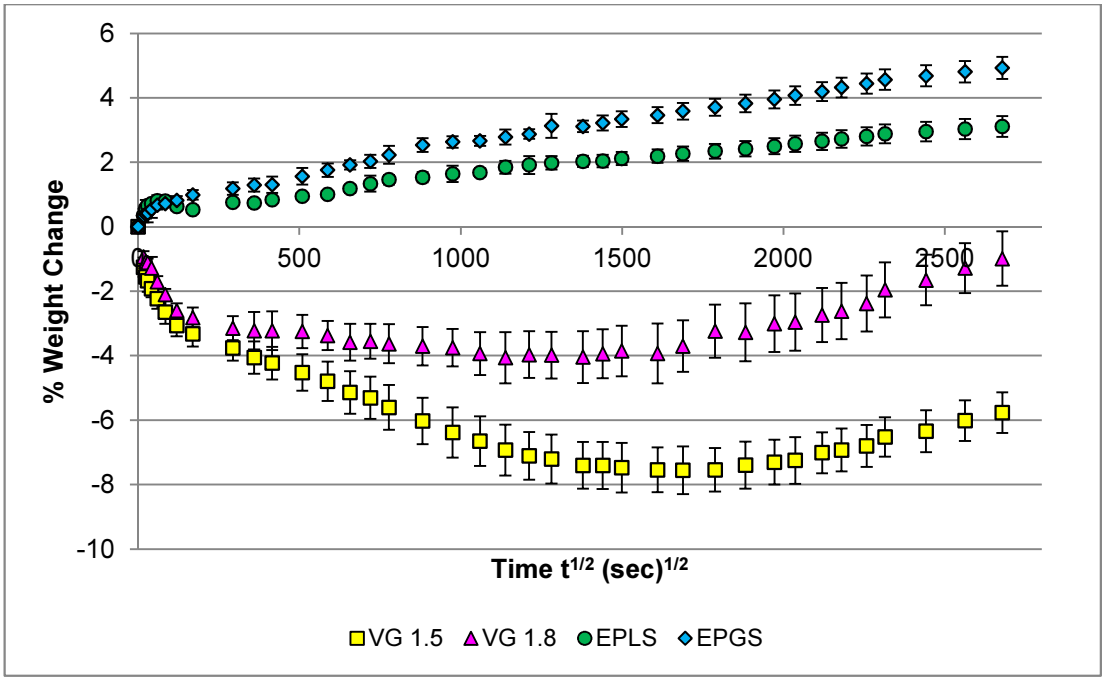

Figure 4.13: Mean (±SD; n=5) % weight change of commercial and experimental tissue conditioner formulations in DW at 37°C for 12 weeks

VG 1.5: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.5g/ml
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

VG 1.8 (commercial control) had the same P/L ratio as EPLS, so this formulation was further studied with respect to addition of CHD and NaF. Figure 4.14 shows the % weight change for VG1.8 containing 1% or 9% CHD with and without the addition of 0.5% NaF, VG 1.8 is included as the control whose results could be compared with the same formulations containing CHD with and without NaF. All formulations showed weight loss. The addition of 1% CHD to the VG 1.8 formulations resulted in increased weight loss; this increase in weight loss was further enhanced with an increase in CHD to 9%. Addition of NaF enhanced the weight loss at both levels of CHD. All formulations lost weight continuously with the exception of VG 1%CHD+F, which started to increase in weight at the end of experiment. Again weight loss is indicative of loss of components, in this case CHD together with the other components.

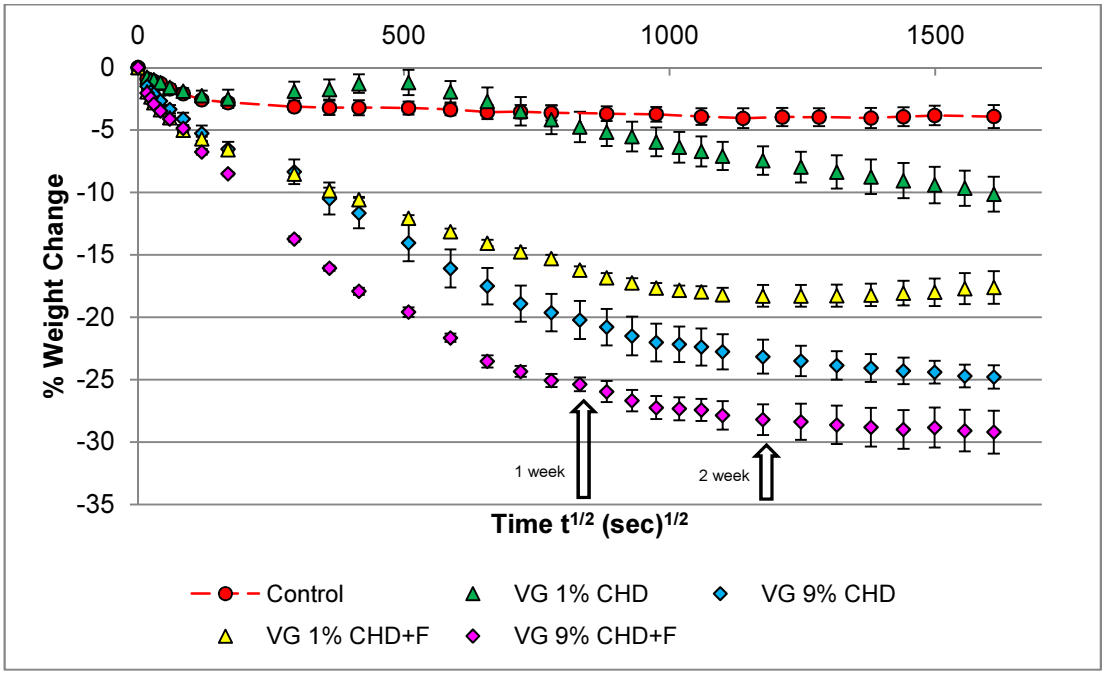

Figure 4.14: Mean (±SD; n=5) % weight change of VG with the addition of 1% or 9% CHD with and without 0.5% NaF, in DW at 37°C for 4 weeks

VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml

Figure 4.15 shows the % weight change of EPLS formulations incorporating 1% or 9% CHD, with and without 0.5% NaF; EPLS is also included as a control. All formulations gained weight throughout the experiment. Although the weight change increased in the 1% and 9% CHD materials compared to control, there was very little difference in weight change between the two (9.5%±0.4 and 8.8%±1.3 respectively); the amount of CHD in EPLS did not appear to have an effect on the weight change. Incorporation of NaF further increased the weight gain of EPLS formulations where 9%CHD+F had a higher weight change (47.6%±0.4) compared to EPLS 1%CHD+F (42%±2.5) at 4 weeks. Hence the lower ethanol content in EPLS reduced the effect on the weight change of increasing CHD from 1% to 9% although inclusion of NaF increased weight change; it had a more significant effect on 9%CHD compared to VG.

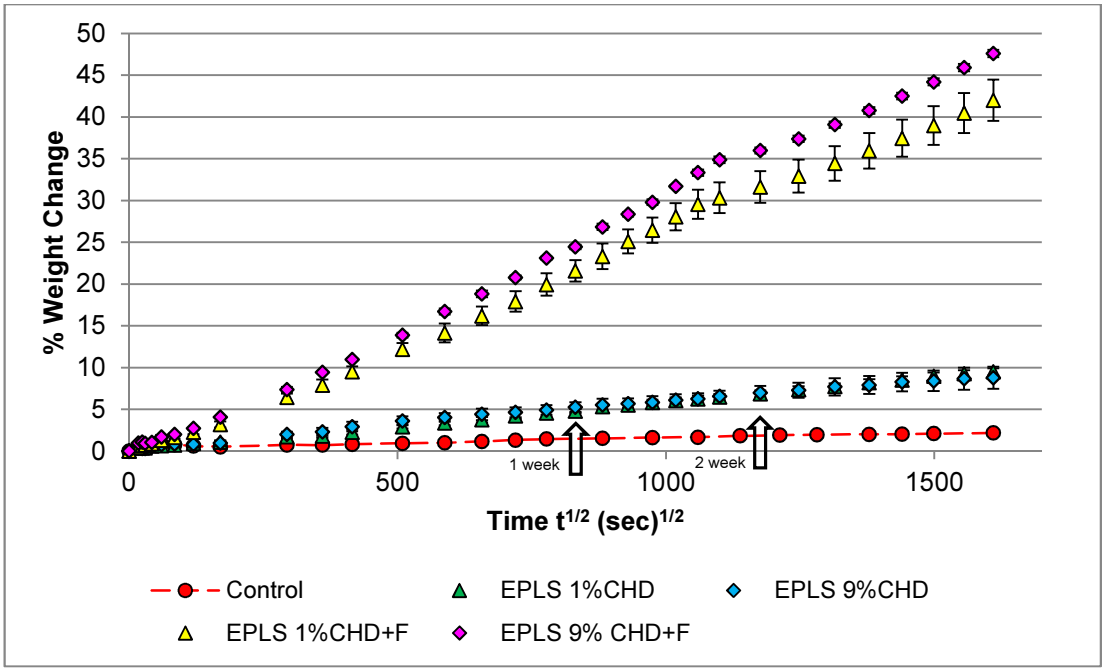

Figure 4.15: Mean (±SD; n=5) % weight change of EPLS with the addition of 1% and 9% CHD with and without 0.5% NaF in DW at 37°C for 4 weeks

EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml

Figure 4.16 shows the % weight change of EPGS incorporating 1% or 9% CHD with and without 0.5% NaF, and also EPGS as the control. The weight gain of all formulations appeared to be linear with the $t^{1/2}$ axis, with the exception of EPGS 1%CHD+F, which was concave to this axis. The data for 1% and 9% CHD+F were plotted against time (Figure 4.17) to determine whether the % weight change were following Case II diffusion; however, the plots proved not to be linear. Closer inspection of Figure 4.16, does show a small initial linear region. Therefore initial uptake kinetics were Fickian followed by anomalous behaviour. As before weight gain was enhanced when 1% CHD was added to the control and it was further increased with the 9% CHD addition. Incorporation of NaF led to a further increase in weight gain for both 1% and 9% CHD formulation.

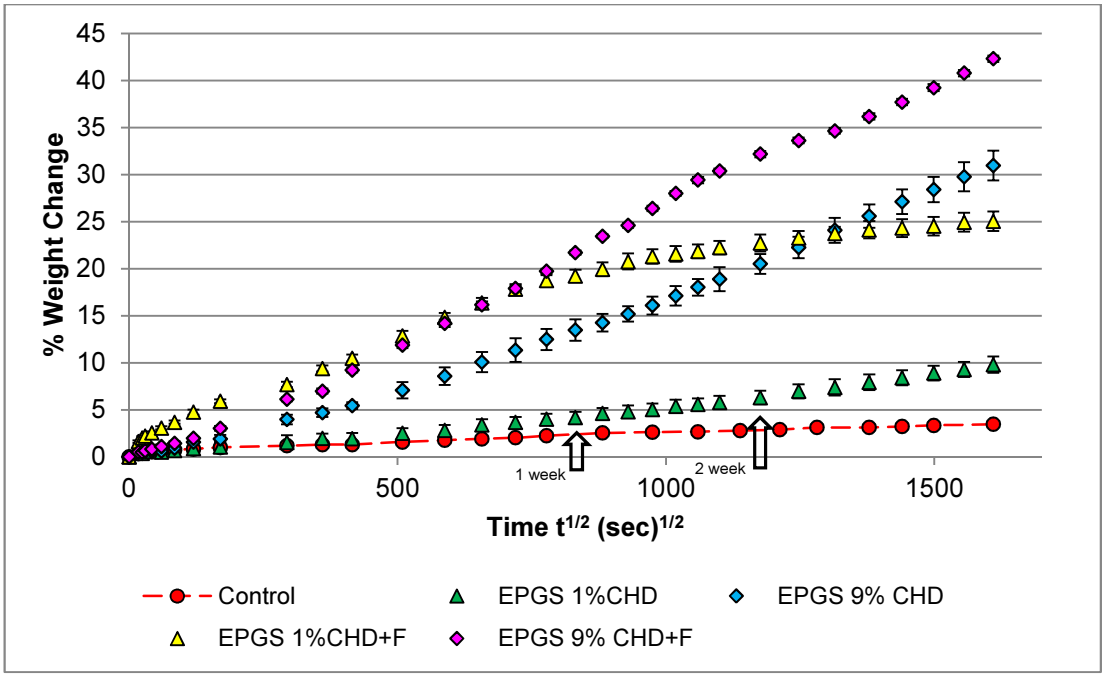

Figure 4.16: Mean (±SD; n=5) % weight change of EPGS with the addition of 1% and 9% CHD with and without 0.5% NaF in DW at 37°C for 4 week

EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

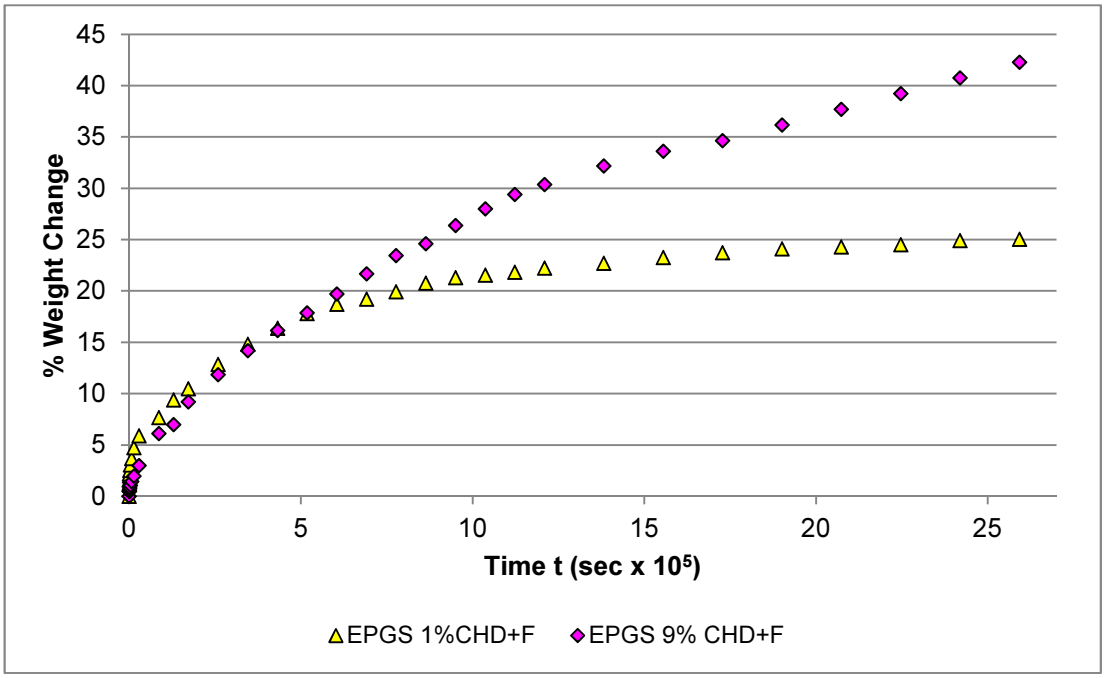

Figure 4.17: Mean (n=5) % weight change data of EPGS 1% & 9% CHD+F plotted against time in sec x10^5

Figures 4.18 and 4.19 show the comparison of the % weight change of tissue conditioner formulations containing 1% CHD with or without 0.5% NaF and 9% CHD with or without 0.5% NaF respectively. In both figures the effect of additives can be clearly seen. When CHD is added to the controls the weight loss/gain was increased which was amplified when NaF was incorporated along with CHD. In most cases the addition of NaF enhanced the % weight change in formulations with 1% CHD to a greater extent than the 9% CHD formulations.

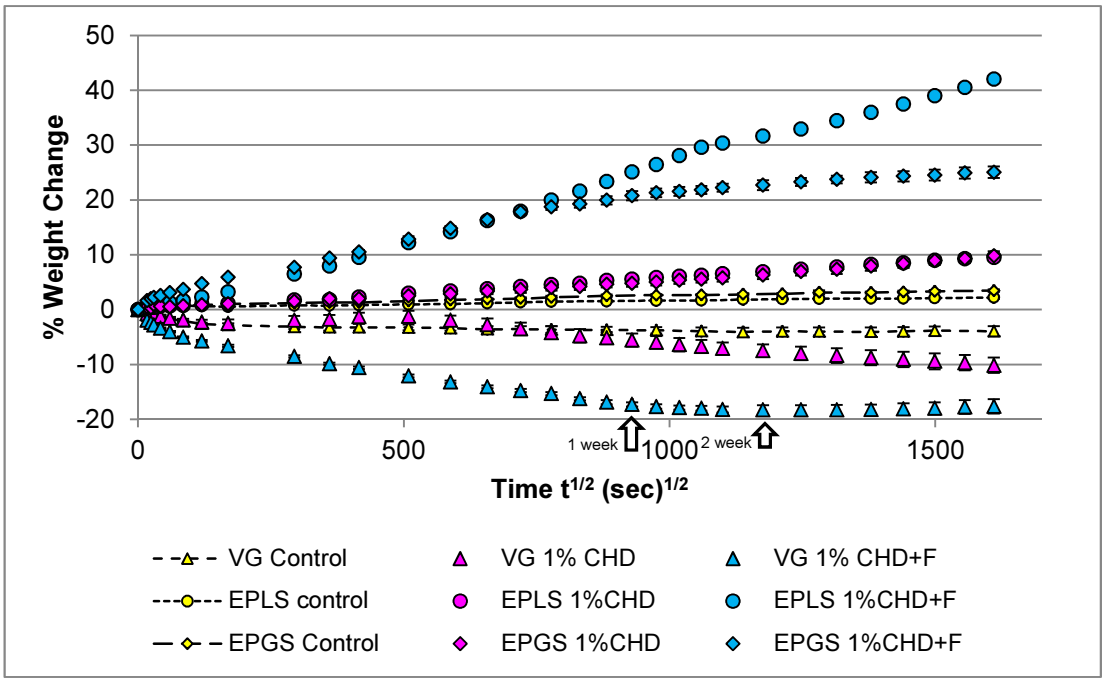

Figure 4.18: Mean (±SD; n=5) % weight change of 1%CHD tissue conditioner formulations with and without 0.5% NaF in DW at 37°C for 4 weeks

VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

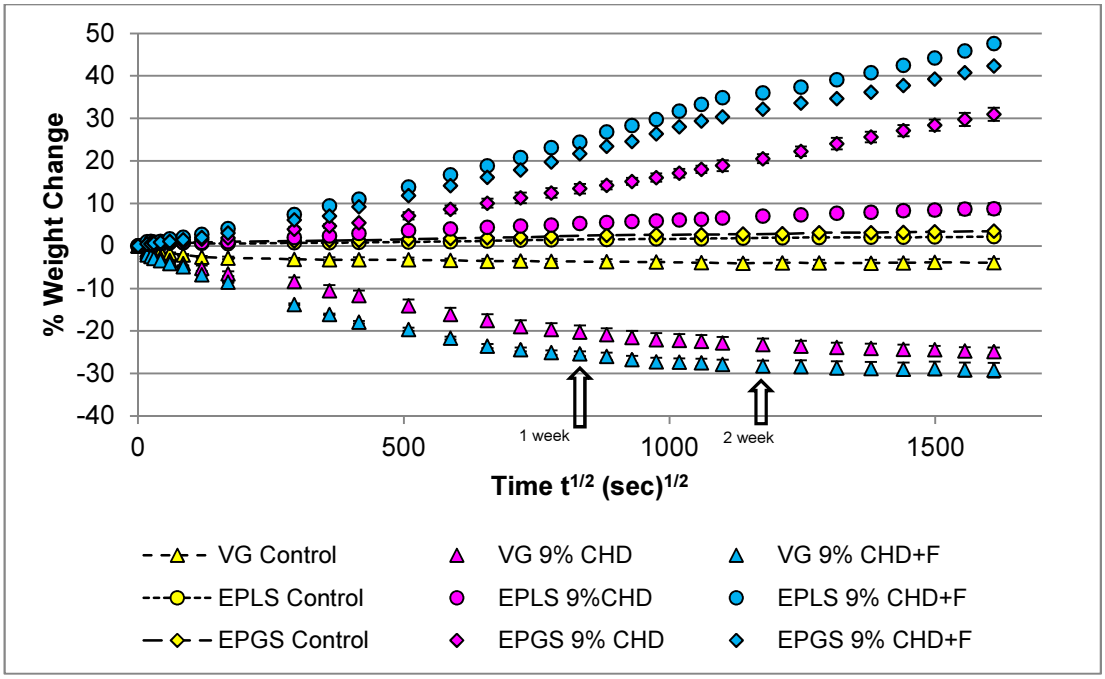

Figure 4.19: Mean (±SD; n=5) % weight change of 1%CHD tissue conditioner formulations with and without 0.5% NaF in DW at 37°C for 4 weeks

VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

4.5 Water Desorption

Although all formulations did not reach equilibrium after water absorption for 12 weeks (for formulations without additives) and 4 weeks (for formulations with additives), the specimens were desorbed at 37°C, as described in section 3.2.9 (page 121), for a period of 1 week to allow equilibrium to be reached. The results are presented as graphs of % weight loss against square root of time. The results are usually presented in positive weight loss but here they are presented in negative weight loss because VG formulations showed a weight loss during the uptake experiment. Therefore for consistency purposes they are shown in negative values. All tissue conditioner formulations showed a similar trend of a rapid initial weight loss which then reached a state of equilibrium. Clearly, desorption was much faster than the absorption process, with all systems reaching equilibrium within a week.

Figure 4.20 shows the % weight loss of VG, VG Old, EPLS and EPGS tissue conditioner formulations. VG Old, EPGS and EPLS reached equilibrium after 8 hours with weight losses of 9%±0.4 for VG Old 1.3, 4.3%±0.2 for VG Old 1.8, 5.6%±0.2 for EPGS and 4.8%±0.2 for EPLS, whereas the VG 1.5 and VG 1.8 reached equilibrium after 24 hours with an increased weight loss of 15.6%±0.7 and 19.1±0.4 respectively. The latter reflects the higher weight change observed in the absorption process.

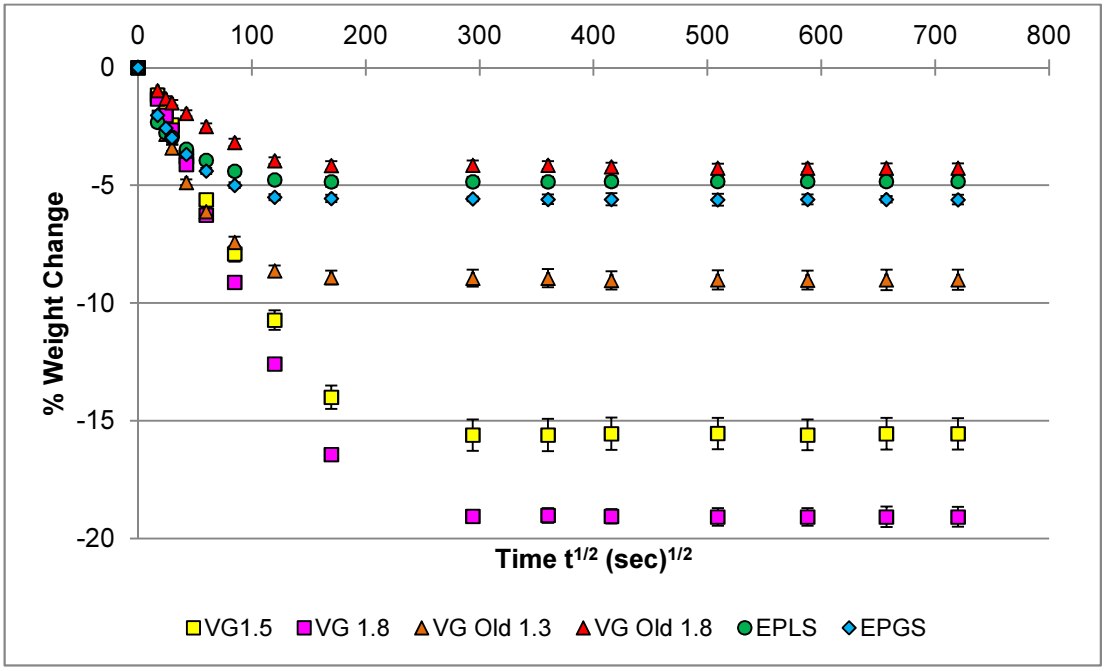

Figure 4.20: Mean (±SD; n=5) % weight loss of tissue conditioner formulations

VG 1.5: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.5g/ml
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
VG Old 1.3: PEMA powder & 93%BPBG+7%ethanol; P/L ratio 1.3g/ml
VG Old 1.8: PEMA powder & 93%BPBG+7%ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

Figure 4.21 shows the % weight loss of VG formulations incorporating 1% or 9% CHD, with and without 0.5% NaF. All formulations, with the exception of VG 9%CHD, showed an initial rapid weight loss over 24 hours after which they reached equilibrium, whereas VG 9% CHD reached equilibrium after 8 hours (13.7%±0.3). The formulation with 1%CHD+F showed the greatest weight loss.

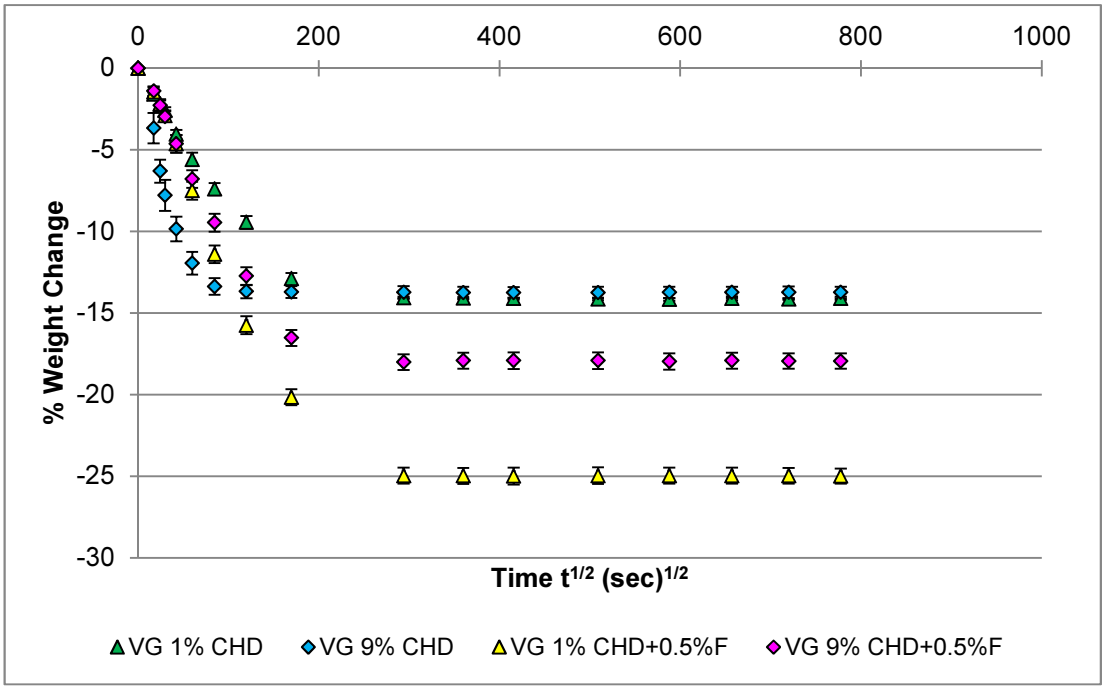

Figure 4.21: Mean (±SD; n=5) % weight loss of VG with the addition of 1% and 9% CHD with and without 0.5% NaF

VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml

Figure 4.22 shows the % weight loss of EPLS formulations incorporating 1% or 9% CHD, with and without 0.5% NaF. All formulations showed an initial rapid weight loss up to 24 hours and then they reached equilibrium. The formulations containing CHD and F showed the greatest weight loss (9%CHD+F = 37.3%±0.2; 1%CHD+F = 35%±0.1).

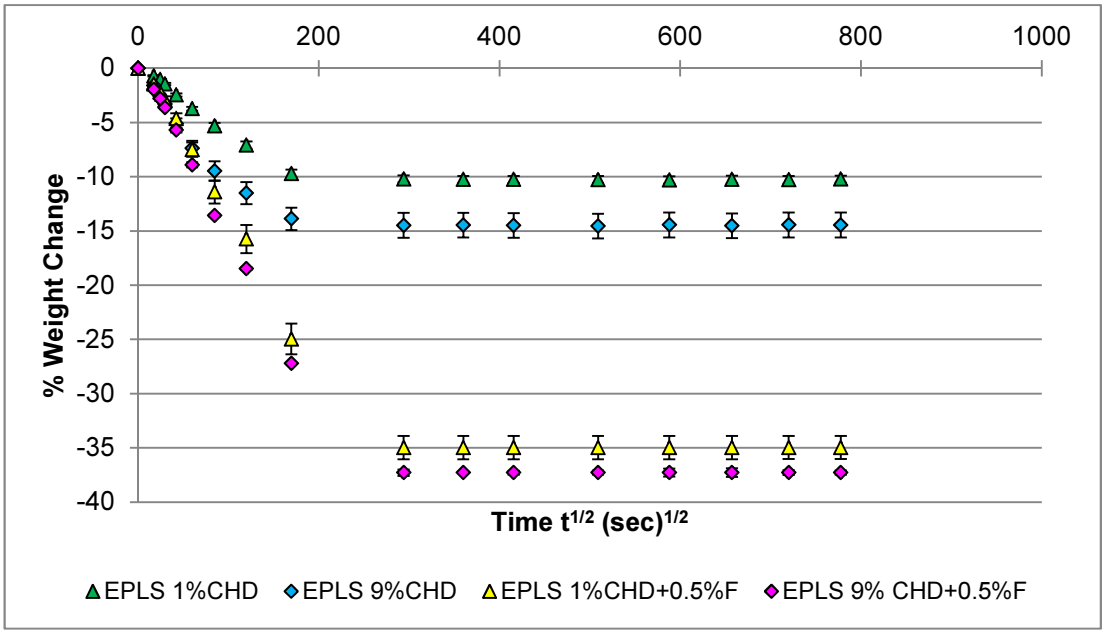

Figure 4.22: Mean (±SD; n=5) % weight loss of EPLS with the addition of 1% and 9% CHD with and without 0.5% NaF

EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml

Figure 4.23 shows the % weight loss of EPGS formulations incorporating 1% or 9% CHD, with and without 0.5% NaF. All formulations showed an initial rapid weight loss up to 24 hours and then they reached equilibrium, except for EPGS 1% CHD, which showed the least weight loss (10.2%±0.3) and reached equilibrium after only 4 hours. EPGS 9% CHD+F showed the greatest weight loss (39.3%±0.3) followed by EPGS 9% CHD (27.8%±0.8) and then EPGS 1% CHD+F (26.3%±1). It should be noted that desorption for all formulations followed Fickian diffusion.

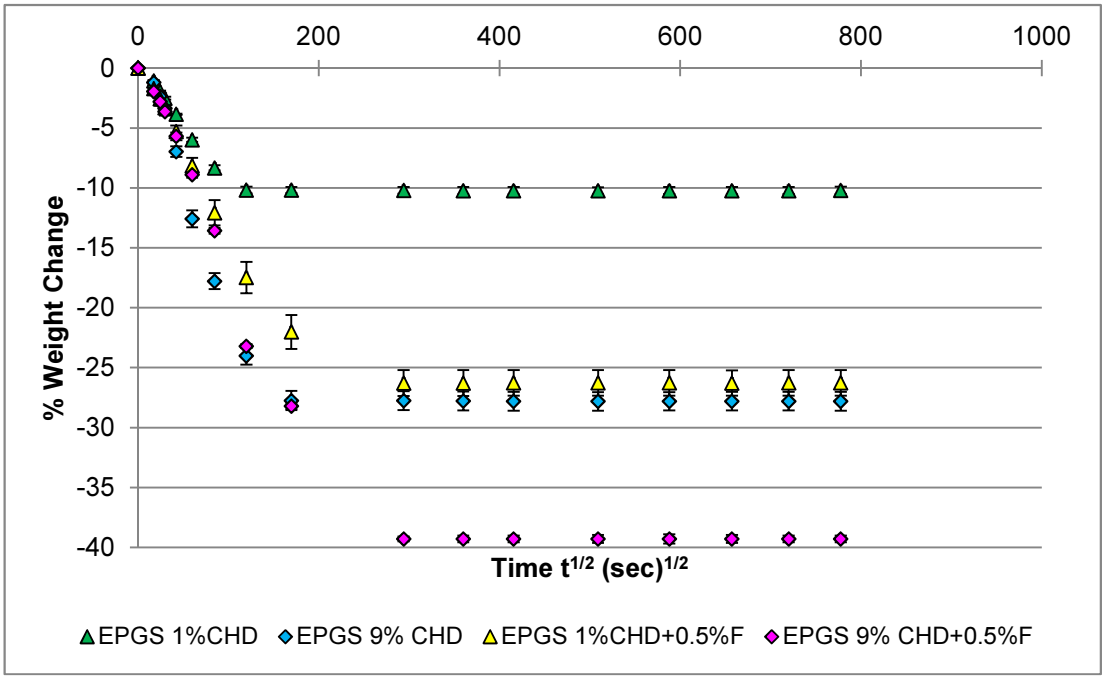

Figure 4.23: Mean (±SD; n=5) % weight loss of EPGS with the addition of 1% and 9% CHD with and without 0.5% NaF

EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

4.6 Solubility & Diffusion Coefficient

The diffusion coefficients for the water absorption process could not be calculated as none of the formulations reached a state of equilibrium, hence only desorption diffusion coefficients were calculated. Figure 4.24 shows an example of a typical graph used to calculate the diffusion coefficient. The slope of the plot was calculated where $M_t/M_\infty \leq 0.5$. The desorption diffusion coefficient (D_{des}) was then calculated using equation 4.7 (Section 3.2.10; page 121).

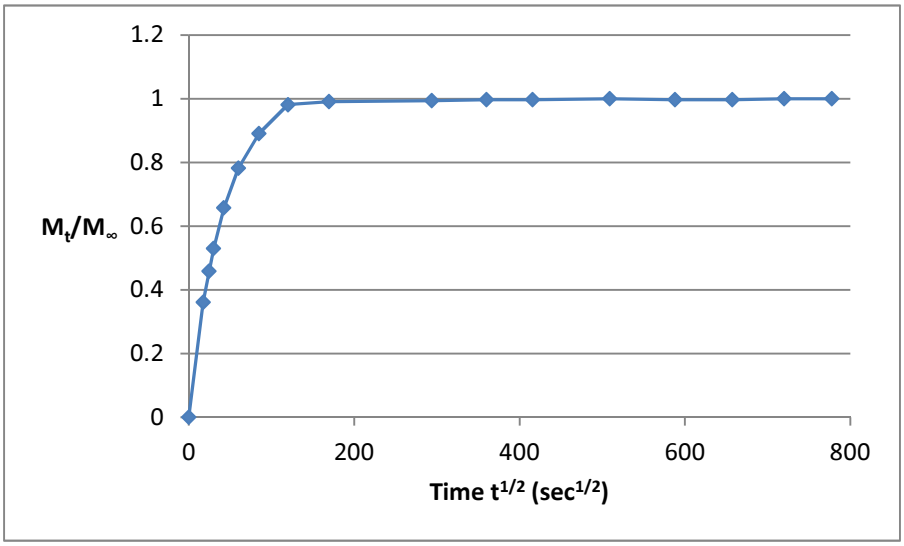

Figure 4.24: M_t/M_∞ graph of EPGS desorption against square root of time

Table 4.3 shows the mean, % uptake weight change, % solubility, % real uptake and D_{des} data for the different tissue conditioner formulations. These were calculated using equations 4.4 to 4.7 (Section 3.2.10; page 121). All the values increased when P/L ratio of VG was increased from 1.5 to 1.8, except % solubility where no significant difference ($p \leq 0.05$) was found, with the exception of VG Old. Interestingly VG Old 1.3 gave a negative solubility result. This will be discussed in section 5.4.

Higher % solubility and % real uptake values also indicated more loss of components from the material. The diffusion coefficients for VG were significantly lower compared to EPLS and EPGS formulations. It appears that a change in plasticiser decreased the D_{des} from 10^{-10} m^2sec^{-1} for VG Old to 10^{-11} m^2sec^{-1} for VG.

Table 4.3: Mean (±SD; n=5), % uptake weight change, % solubility, % real uptake and desorption diffusion coefficient data of different tissue conditioner formulations for 12 weeks

Formulation	% uptake Weight Change	% Solubility	% Real Uptake	D_{des} (m^2sec^{-1})
VG 1.5	-5.77 ± 0.6	19.7 ± 1.6[a]	14.0 ± 2.1	2.56 x 10^{-11}
VG 1.8	-0.99 ± 0.8	20.2 ± 2.1[a]	19.3 ± 2.6	1.96 x 10^{-11}
VG Old 1.3	9.13 ± 0.9	-4.5 ± 0.3	4.7 ± 0.4[a]	1.03 x 10^{-10}
VG Old 1.8	1.30 ± 0.2	3.0 ± 0.1[b]	4.3 ± 0.3[a]	1.40 x 10^{-10}
EPLS	3.11 ± 0.3	1.9 ± 0.4[b]	5.0 ± 0.6[a]	5.59 x 10^{-10}
EPGS	4.93 ± 0.3	1.0 ± 0.4[b]	5.9 ± 0.5[a]	3.14 x 10^{-10}

(No significant difference between groups with same letters; p≤0.05)

Table 4.4 shows the mean % uptake weight change, % solubility, % real uptake and D_{des} data of different tissue conditioner formulations incorporating 1% or 9% CHD, with and without 0.5% NaF. Generally, and as expected, the % solubility increased with the addition of CHD, and when this was increased from 1% to 9% in all formulations. The addition of NaF also significantly increased the % solubility in all

the formulations except EPLS 9%CHD. VG formulations had significantly higher % solubility values compared to EPGS and EPLS. The opposite effect of % solubility can be seen for D_{des} where increasing the CHD percentage from 1% to 9% decreased D_{des}. Addition of 0.5% NaF also led to lower D_{des} values.

Table 4.4 Mean (±SD; n=5), % uptake weight change, % solubility, % real uptake and desorption diffusion coefficient data of different tissue conditioner formulations incorporating 1% or 9% CHD, and with or without 0.5% NaF for 4 weeks

Formulation	% Uptake Weight change	% Solubility	% Real Uptake	D_{des} (m^2sec^{-1})
VG+1%CHD	-10.15 ± 1.4	22.8 ± 1.1	$12.7 \pm 0.1^{+}$	3.49×10^{-11}
VG+9%CHD	-24.80 ± 0.9	35.1 ± 1.0	$10.3 \pm 0.2^{-}$	1.40×10^{-10}
VG+1%CHD+F	-17.62 ± 1.3	38.2 ± 2.0	20.6 ± 0.9	1.55×10^{-11}
VG+9%CHD+F	-29.21 ± 1.7	41.9 ± 2.8	$12.7 \pm 1.1^{+}$	2.56×10^{-11}
EPLS+1%CHD	9.51 ± 0.4^{a}	1.7 ± 0.2^{A}	$11.2 \pm 0.4^{+-}$	2.56×10^{-11}
EPLS+9%CHD	8.78 ± 1.3^{a}	7.0 ± 0.3^{BC}	15.7 ± 1.4	5.03×10^{-11}
EPLS+1%CHD+F	41.99 ± 2.5^{b}	7.7 ± 0.5^{C}	49.7 ± 1.2	1.16×10^{-11}
EPLS+9%CHD+F	47.60 ± 0.4	7.4 ± 0.9^{BC}	55.0 ± 1.4	1.26×10^{-11}
EPGS+1%CHD	9.79 ± 0.9^{a}	1.5 ± 0.5^{A}	$11.3 \pm 0.3^{+-}$	5.03×10^{-11}
EPGS+9%CHD	30.96 ± 1.6	5.5 ± 0.4^{B}	36.4 ± 1.5	2.23×10^{-11}
EPGS+1%CHD+F	25.04 ± 1.0	7.8 ± 0.5^{C}	32.8 ± 0.4	1.96×10^{-11}
EPGS+9%CHD+F	42.30 ± 0.3^{b}	10.8 ± 0.9	53.1 ± 1.2	1.04×10^{-11}

(No significant difference between groups with same letters; p≤0.05)
Univariate Analysis of Variance to check the relationship between materials (VG, EPLS & EPGS), CHD conc. (1% & 9%) with or without NaF showed highly significant (p≤0.05) among the groups.

4.7 Chlorhexidine Release

Prior to measuring the amount of CHD released from specimens, first a calibration curve was plotted as shown in Figure 4.25. From the slope of the line (y = 42.97x - 0.0157) the amount of CHD released at each time interval, as described in section 3.2.11 (page 122), was calculated. The results are shown as plots of % CHD release against time in seconds and/or square root of time to identify possible release mechanisms involved.

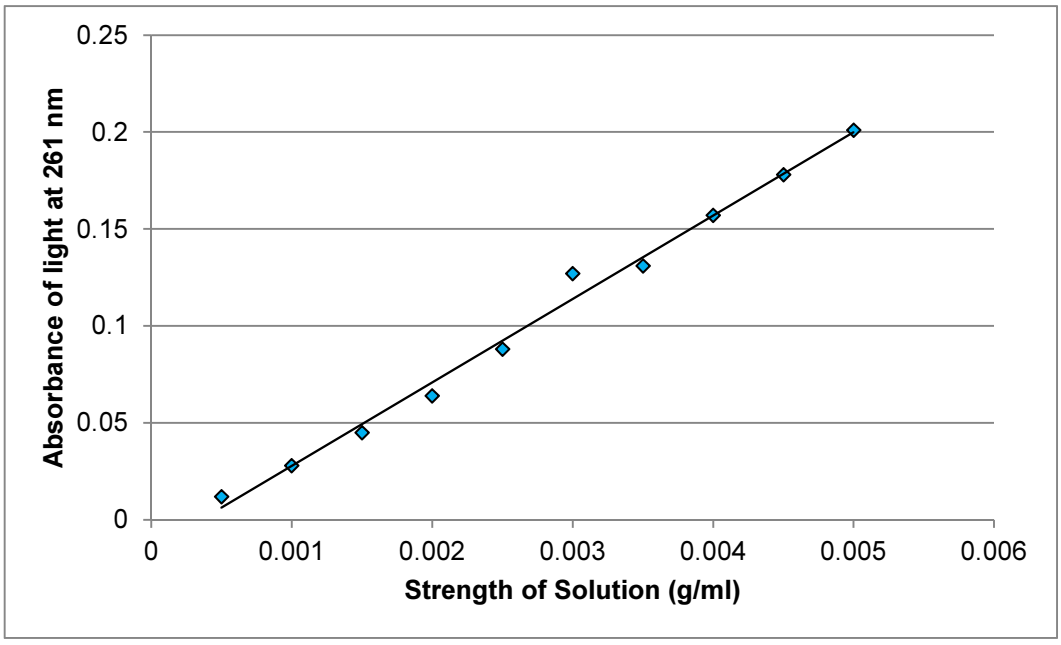

Figure 4.25: Calibration curve for chlorhexidine measurement

Figures 4.26 and 4.27 show the % CHD release from VG 1% and 9% CHD, with and without the addition of 0.5% NaF. The amount of CHD released was reduced (47.3%±0.9 to 8%±2.5) at the end of 4 weeks with increasing CHD in the formulation from 1% to 9%. Incorporation of NaF in the systems increased the % release of CHD in both 1% and 9% CHD formulations. NaF had a greater effect on the release of CHD in the 1% CHD formulation; it increased its release by ~30% at the end of the experimental time period.

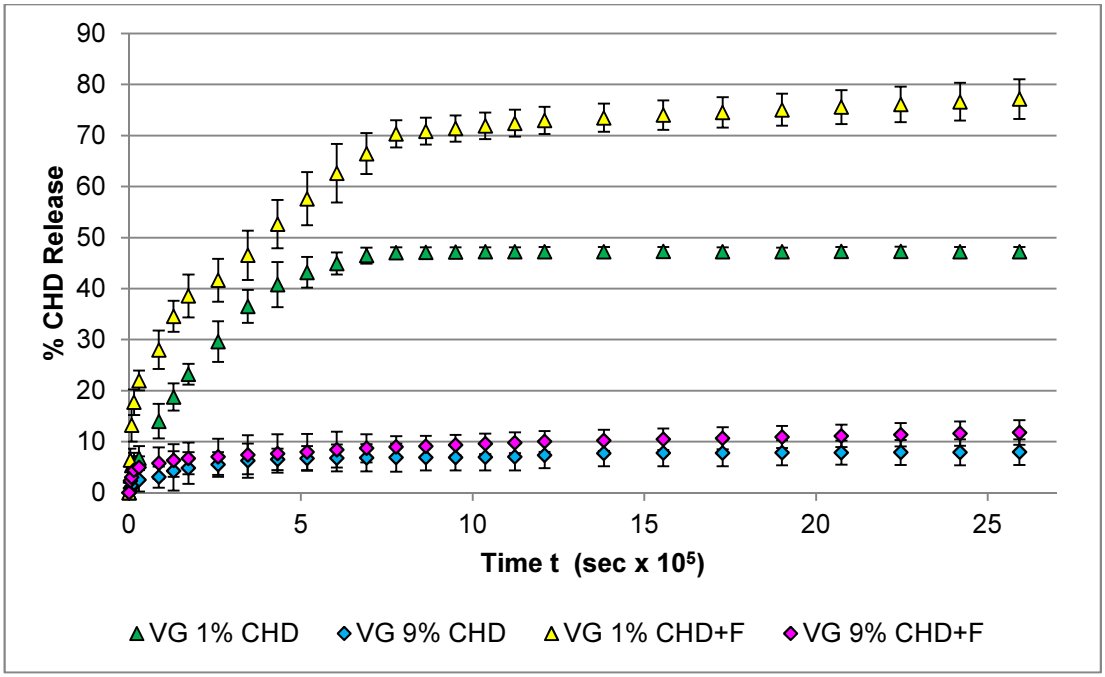

Figure 4.26:Mean (±SD; n=5) % CHD release of VG 1% & 9% with and without the addition of 0.5% NaF in DW at 37ºC for 4 weeks plotted against time in sec

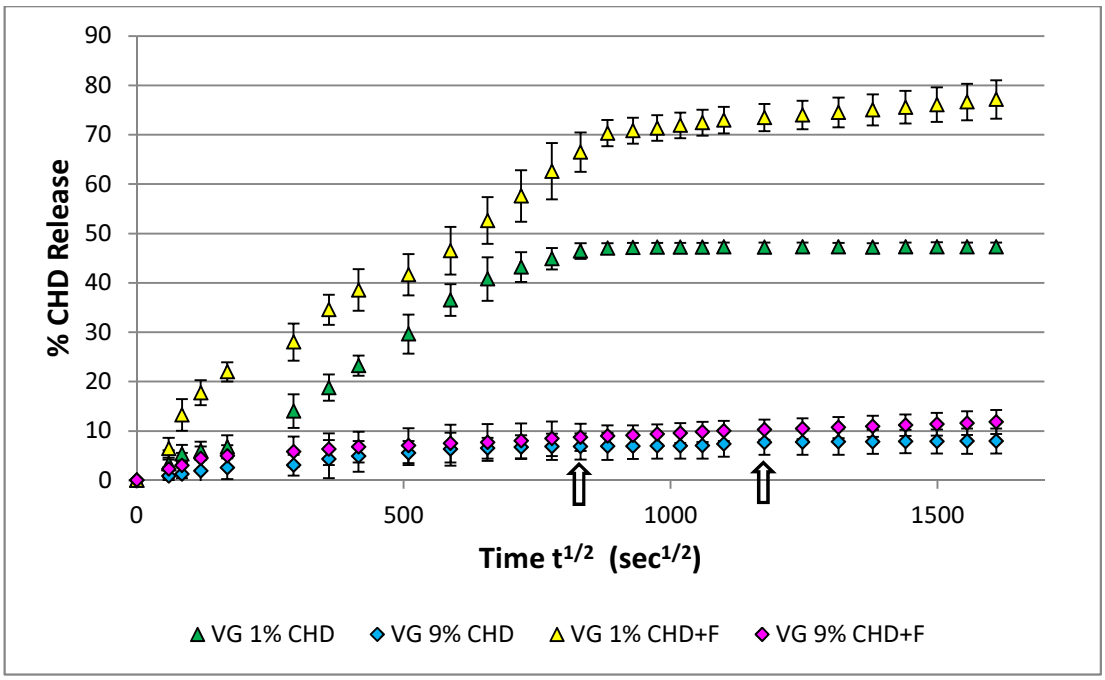

Figure 4.27: Mean (±SD; n=5) % CHD release of VG 1% & 9% with and without the addition of 0.5% NaF in DW at 37ºC for 4 weeks plotted against square root of time in sec$^{1/2}$

VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml

Figures 4.28 and 4.29 show the release of CHD (%) from EPLS containing 1% and 9%CHD, with and without 0.5% NaF. Here again EPLS containing 1% CHD released more CHD (50.3±0.1) compared with EPLS 9%CHD (20.2± 0.3). Addition of NaF enhanced the CHD release which was more significant in EPLS 1% CHD+ F (78.9±0.2) than in EPLS 9% CHD+F (27.2±0.3). Also there was an initial rapid release seen in both formulations with NaF followed by a slower release. From Figure 4.29 it can be seen that by incorporating NaF the release profiles/ mechanisms have been changed.

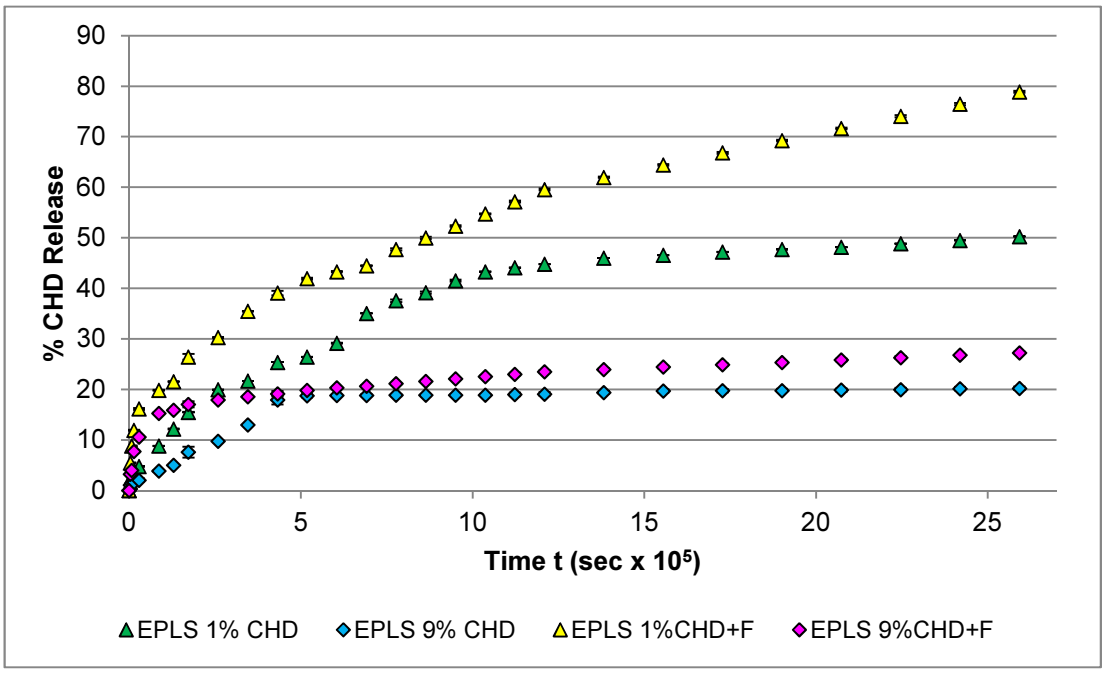

Figure 4.28: Mean (±SD; n=5) % CHD release of EPLS 1% & 9% CHD with and without the addition of 0.5% NaF in DW at 37ºC for 4 weeks against time in sec

EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml

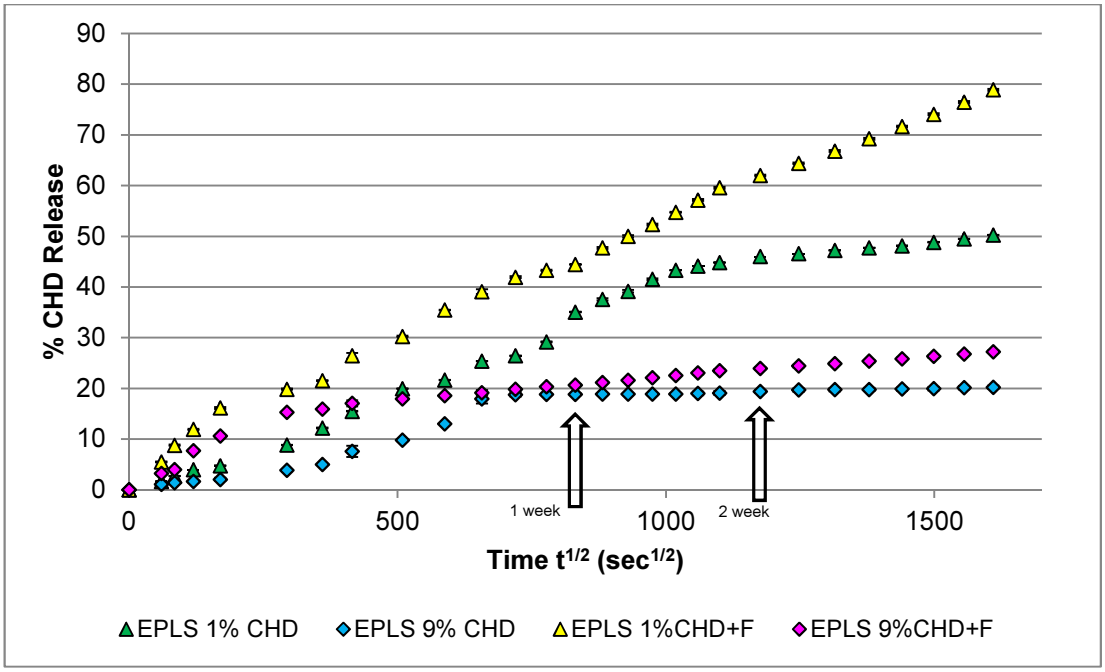

Figure 4.29: Mean (±SD; n=5) % CHD release of EPLS 1% & 9% CHD with and without the addition of 0.5% NaF in DW at 37ºC for 4 weeks against square root of time in sec$^{1/2}$

EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml

Figure 4.30 and 4.31 show the % release of EPGS formulations containing 1% and 9% CHD with and without 0.5% NaF. Again EPGS 1% CHD showed more release (5.7%±0.6) compared to EPGS 9% (2.6%±0.1). When NaF was added the release of CHD was further increased from both formulations (21.7%±0.4 and 3.9%±0.3 in EPGS 1%CHD+F and EPGS 9% CHD+F respectively). NaF had a more substantial effect on the formulation with 1% CHD release than the 9% CHD. In EPGS 1%CHD+F there was an initial rapid release of CHD in the first 8 hours ($t=0.288x10^5$; $t^{1/2}=169.71$) followed by a slower steady release. EPGS 9%CHD+F showed a similar release to EPGS 9%CHD up to day 10 ($t=8.64x10^5$; $t^{1/2}=929.52$) and from this point onwards the former started to show a higher % CHD release up to the end of experimental time period of 4 weeks.

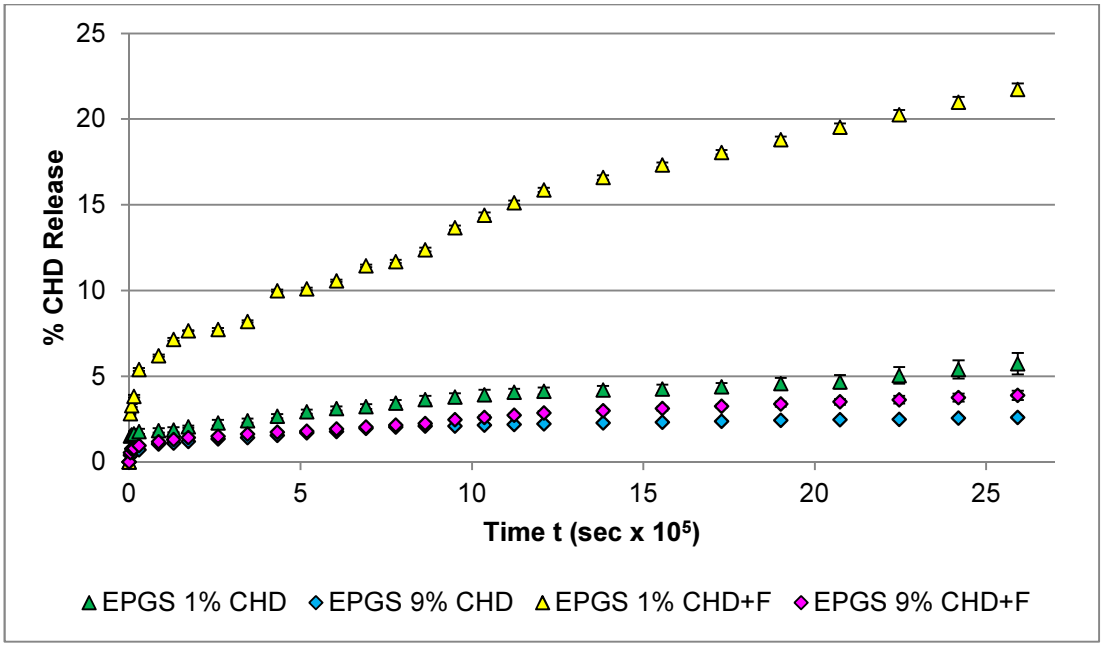

Figure 4.30: Mean (±SD; n=5) % CHD release of EPGS 1% & 9% CHD with and without the addition of 0.5% NaF in DW at 37ºC for 4 weeks plotted against time in sec

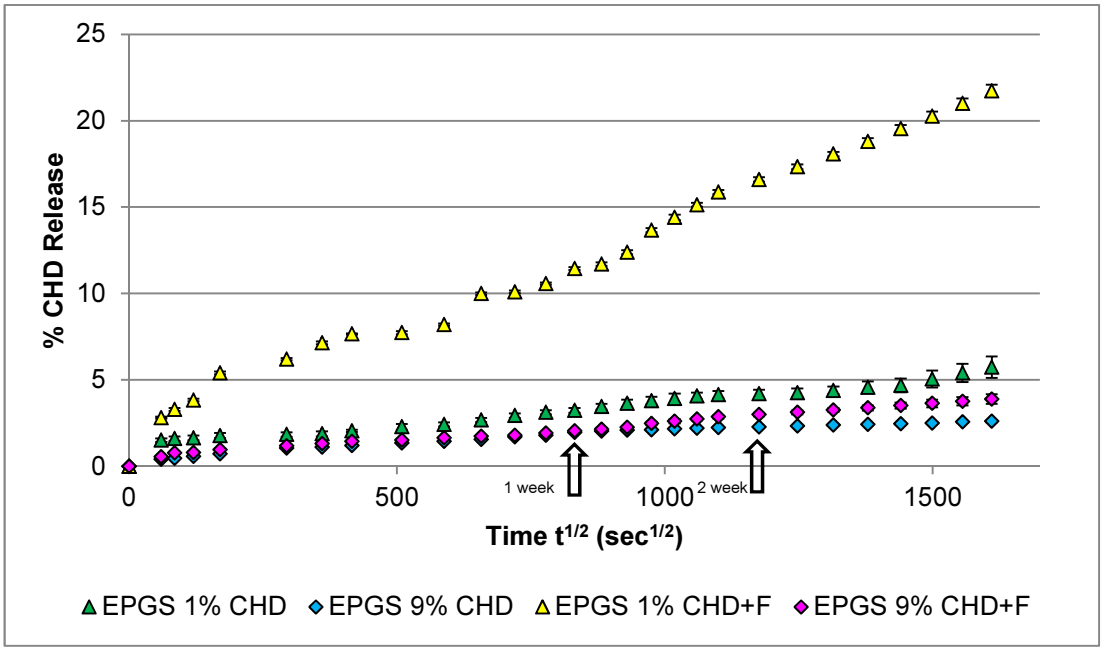

Figure 4.31: Mean (±SD; n=5) % CHD release of EPGS 1% & 9% CHD with and without the addition of 0.5% NaF in DW at 37ºC for 4 weeks plotted against square root of time in sec$^{1/2}$

EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

Figures 4.32 and 4.33 shows the comparison of % CHD release in formulations containing 1% CHD with and without NaF; Figures 4.34 and 4.35 shows the formulations with 9%CHD with and without NaF. Figures 4.32 to 4.35 clearly show that incorporation of NaF enhanced the % release of CHD in all formulations; however it is more noticeable in the 1%CHD formulations. It can also be seen that the EPLS formulations (containing 5% ethanol) showed the highest % release whereas VG formulations containing a higher ethanol content (Appendix A1) had a lower % release and the release was further reduced in EPGS where no ethanol was used, thus highlighting the effect of ethanol content.

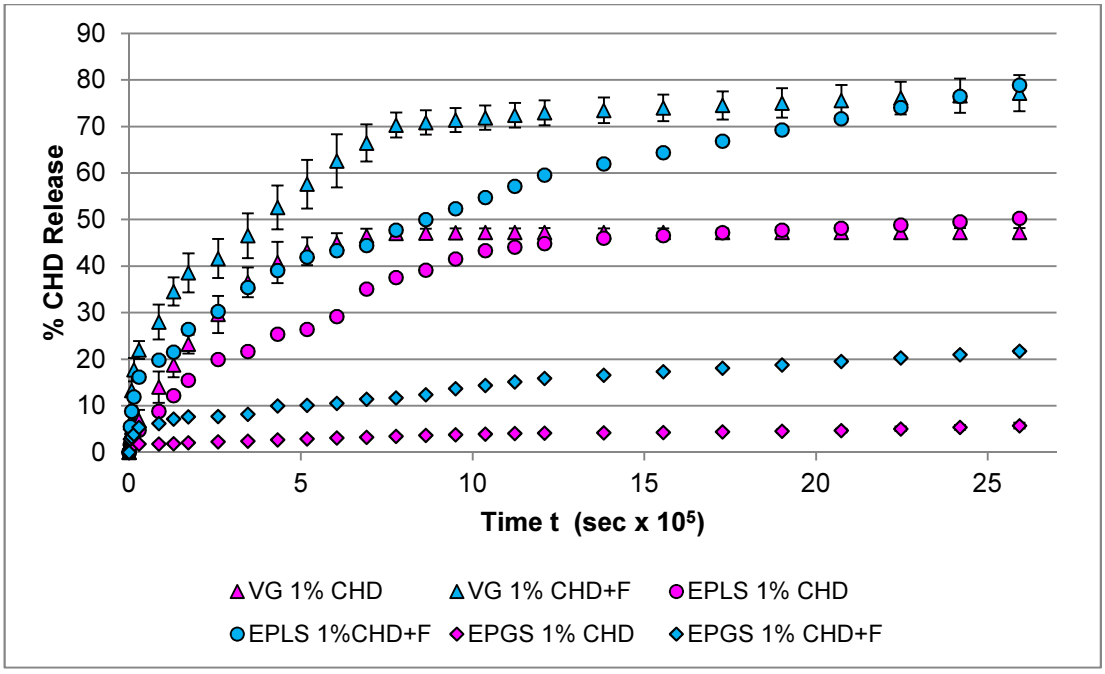

Figure 4.32: Mean (±SD; n=5) % CHD release of tissue conditioner formulations containing 1% CHD with and without the addition of 0.5% NaF in DW at 37ºC for 4 weeks plotted against time in sec

VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

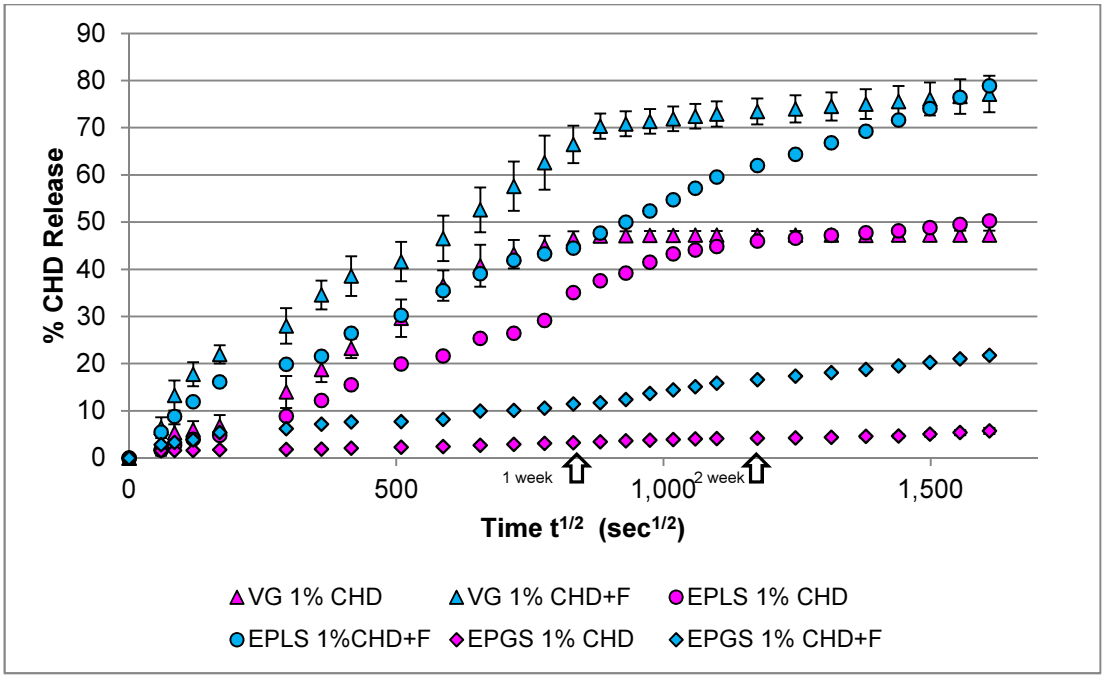

Figure 4.33: Mean (±SD; n=5) % CHD release of tissue conditioner formulations containing 1% CHD with and without the addition of 0.5% NaF in DW at 37ºC for 4 weeks plotted against square root of time in sec$^{1/2}$

VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

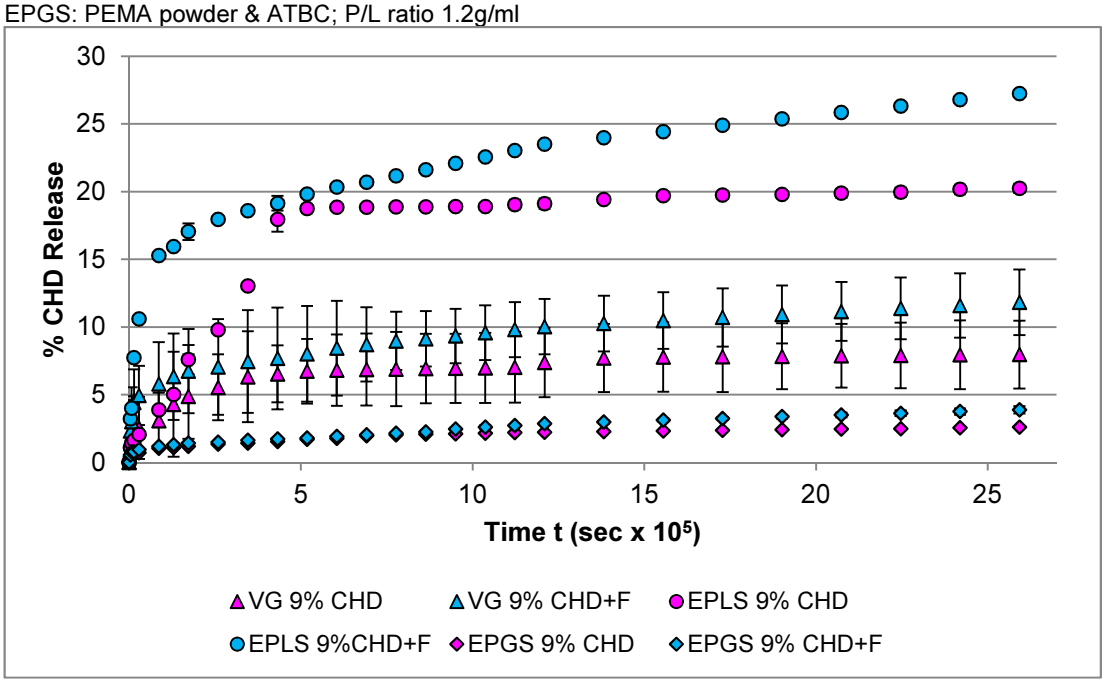

Figure 4.34: Mean (±SD; n=5) % CHD release of tissue conditioner formulations containing 9% CHD with and without the addition of 0.5% NaF in DW at 37ºC for 4 weeks plotted against time in sec

VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

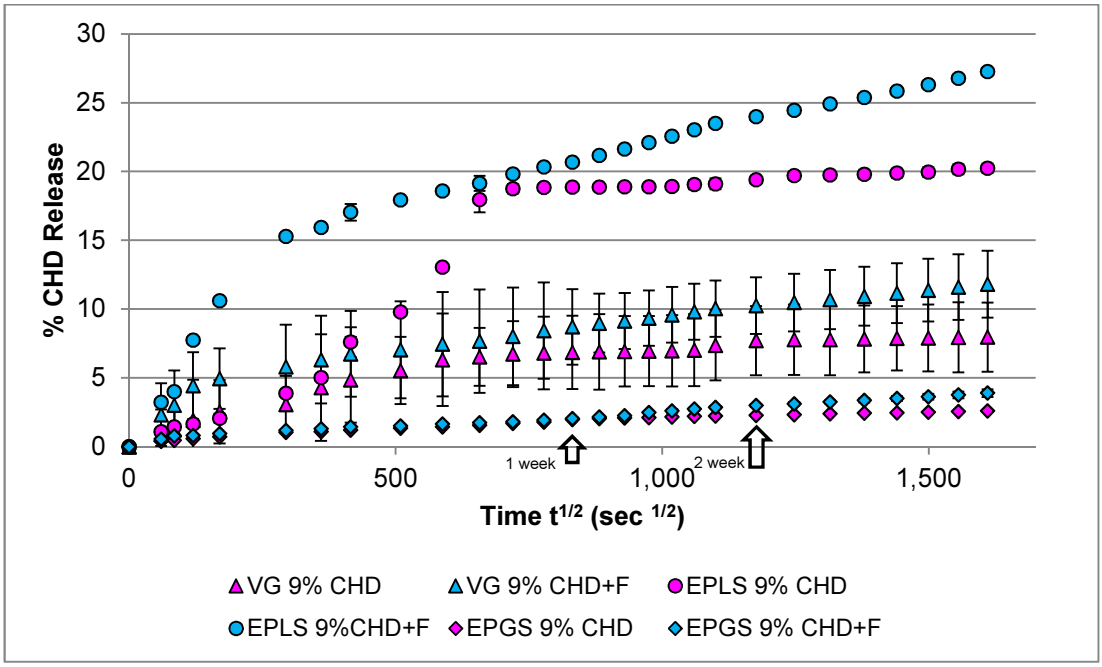

Figure 4.35: Mean (±SD; n=5) % CHD release of tissue conditioner formulations containing 9% CHD with and without the addition of 0.5% NaF in DW at 37ºC for 4 weeks plotted against square root of time in sec$^{1/2}$

VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

Generally the CHD release was in three phases as shown in Figure 4.36. A rapid burst seen in the first 24 hours, then a constant steady release up to 1 week, followed by a slower release rate from weeks 2 to 4. These time periods were used to calculate (using the equation of slope of the line y=mx+b) the CHD release rate per hour in first 24 hours and then as per day in 1st week and the last 3 weeks.

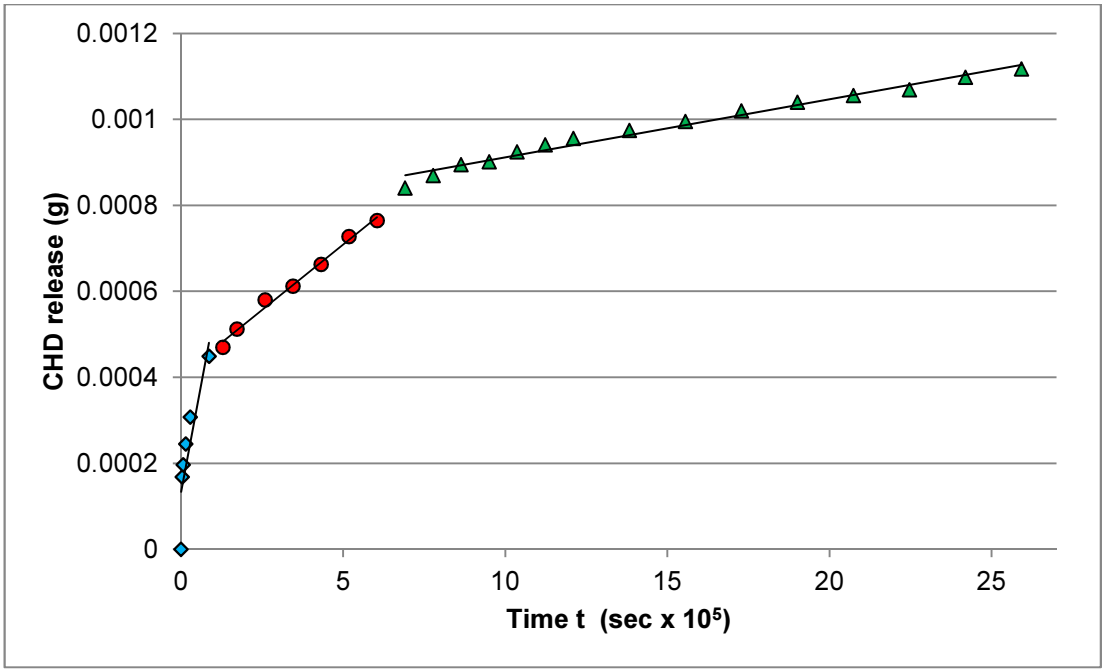

Figure 4.36: CHD release in EPGS 9%CHD showing release in first 24 hours (blue), 1 week (red) and week 2-4 release (green)

Table 4.5 summarizes the CHD release at 24 hours, 1 week and 4 week time. Generally the 1% CHD formulations showed more % release than the 9% CHD formulations but in terms of weight, 9% CHD formulations showed greater release. In all formulations more than 50% of CHD in total was released within the 1st week. Later in 2-4 weeks a smaller quantity of CHD was released, especially in formulations not containing NaF.

Table 4.5: Summary of CHD release in % and in mg in different formulations at different time periods

Formulations	CHD Release	1 Day	1 week	4 week
VG 1%CHD	in %	14.0^{A}	$46.5^{@}$	47.3
	in mg	0.690	2.286	2.326
VG 9%CHD	in %	$3.1^{B,D,E}$	6.9^{+}	8.0^{a}
	in mg	1.359	3.036	3.527
VG 1%CHD+F	in %	28.0	66.5	77.1^{b}
	in mg	1.158	3.459	4.014
VG 9%CHD+F	in %	$5.8^{C,E}$	8.7^{+}	11.8
	in mg	2.717	4.080	5.536
EPLS 1%CHD	in %	8.8^{C}	35.1	50.3
	in mg	0.493	1.955	2.802
EPLS 9%CHD	in %	$3.9^{B,D,E}$	$18.9^{\sim}$	20.2^{c}
	in mg	1.775	8.639	9.274
EPLS 1%CHD+F	in %	19.9	$44.5^{@}$	78.9^{b}
	in mg	1.111	2.489	4.416
EPLS 9%CHD+F	in %	15.3^{A}	$20.7^{\sim}$	27.2
	in mg	8.629	11.680	15.388
EPGS 1%CHD	in %	1.8^{B}	$3.2^{\#}$	$5.7^{a,d}$
	in mg	0.089	0.157	0.278
EPGS 9%CHD	in %	$1.0^{B,D}$	$2.0^{\#}$	2.6^{e}
	in mg	0.449	0.840	1.117
EPGS 1%CHD+F	in %	$6.2^{C,D,E}$	11.4	21.7^{c}
	in mg	0.396	0.730	1.387
EPGS 9%CHD+F	in %	1.2^{B}	$2.0^{\#}$	$3.9^{d,e}$
	in mg	0.651	1.130	2.151

(No significant difference between groups with same letters; $p \leq 0.05$)
Univariate Analysis of Variance to check the relationship between materials (VG, EPLS & EPGS), CHD conc. (1% & 9%) with or without NaF showed highly significant ($p \leq 0.05$) among the groups.

From the graphs of %CHD release against $t^{1/2}$ of the different tissue conditioner formulations the gradients, after 24 hours and associated intercepts of the y-axis were calculated and shown in Table 4.6. The gradients show the rate of diffusion controlled release while the intercepts are an indication of the surface burst release. Negative values of the intercept on y-axis show absence of burst release. Generally

the VG formulations had the highest release rates (gradients) followed by EPLS and then EPGS.

Table 4.6: Gradients & intercepts of plots (%CHD release vs square root of time after 24 hours to 1 week) of % CHD release of tissue conditioner formulations containing 1% and 9% CHD with and without the addition of 0.5% NaF

Formulation	% CHD release gradients	% CHD release intercept
VG+1%CHD	0.0604	-1.2659
VG+9%CHD	0.0055	2.6369
VG+1%CHD+F	0.0678	8.8685
VG+9%CHD+F	0.0048	4.618
EPLS+1%CHD	0.043	-2.9589
EPLS+9%CHD	0.0373	-7.991
EPLS+1%CHD+F	0.048	2.1709
EPLS+9%CHD+F	0.0097	12.708
EPGS+1%CHD	0.0029	0.8023
EPGS+9%CHD	0.0017	0.4556
EPGS+1%CHD+F	0.0091	3.57
EPGS+9%CHD+F	0.0015	0.7754

Table 4.7 shows a summary of CHD release rate in the different formulations at time periods shown in Figure 4.36. Generally the CHD release up to 1 day was increased with an increase of CHD from 1% to 9% and, this was further enhanced when NaF

was added. From 1 day to 1 week CHD release decreased, even when CHD was increased from 1% to 9%, except for EPGS, where it increased. The addition of NaF enhanced CHD release in all formulations, except for VG 9%CHD. From week 1 to week 4 the CHD release rate was more when CHD was increased from 1% to 9%, except for EPLS and the addition of NaF showed a higher release rate in all formulations except VG 9%CHD to it. Also the release rate of CHD was higher up to 1 week compared to the release rates up to 4 week except for VG 9%CHD.

Table 4.7 Summary of CHD release rate in different formulations at different time periods

Formulations	CHD release rate		
	1 Day (mg/day)	**1 week** (mg/day)	**4 week** (mg/day)
VG 1%CHD	0.69	0.259	0.001
VG 9%CHD	1.36	0.173	0.259
VG 1%CHD+F	1.46	0.259	0.017
VG 9%CHD+F	2.72	0.173	0.060
EPLS 1%CHD	0.49	0.173	0.035
EPLS 9%CHD	1.77	0.089	0.034
EPLS 1%CHD+F	1.11	0.263	0.086
EPLS 9%CHD+F	8.63	0.432	0.173
EPGS 1%CHD	0.09	0.009	0.004
EPGS 9%CHD	0.45	0.052	0.009
EPGS 1%CHD+F	0.39	0.043	0.026
EPGS 9%CHD+F	0.65	0.060	0.043

4.8 Fluoride Release

Fluoride was added to the different formulations containing 1% and 9% CHD in the form of sodium fluoride (NaF). The release of F ions in DW at 37°C was then measured at the same time periods as the CHD release, as described in section 3.2.11 (page 122). The results were plotted as % release of F against time in seconds and square root of time, to study the amount released and identify possible release mechanisms involved. However plots against time were not very helpful so only square root of time plots are shown.

Figures 4.37 and 4.38 show the % F release from VG, EPLS and EPGS with 1% CHD and 9% CHD respectively. In 1% and 9% CHD formulations, VG (containing the highest ethanol content) showed the highest % F release followed by EPLS (containing 5% ethanol) while EPGS (containing no ethanol) showed the lowest release. From Figure 4.37 the effect of ethanol on the release profile is clearly seen, where in the absence of ethanol the release in EPGS is less. VG and EPLS have similar release profiles but different to EPGS, up to 2 weeks ($t^{1/2}$=1099.8). The release slows after 2 weeks ($t^{1/2}$=1099.8) for both EPLS and EPGS whereas little or no effect is seen in VG formulations (Figure 4.37). A similar effect was seen in formulations with 9% CHD (Figure 4.38), where the F release slowed in all formulations after 2 weeks ($t^{1/2}$=1099.8), more noticeably for EPLS and EPGS than VG. Figure 4.38 shows that all three materials containing 9% CHD have similar release profiles up to 24 hours ($t^{1/2}$=1099.8). Release then diverges with VG releasing the highest amount followed by EPLS and then EPGS, again showing the effect of ethanol.

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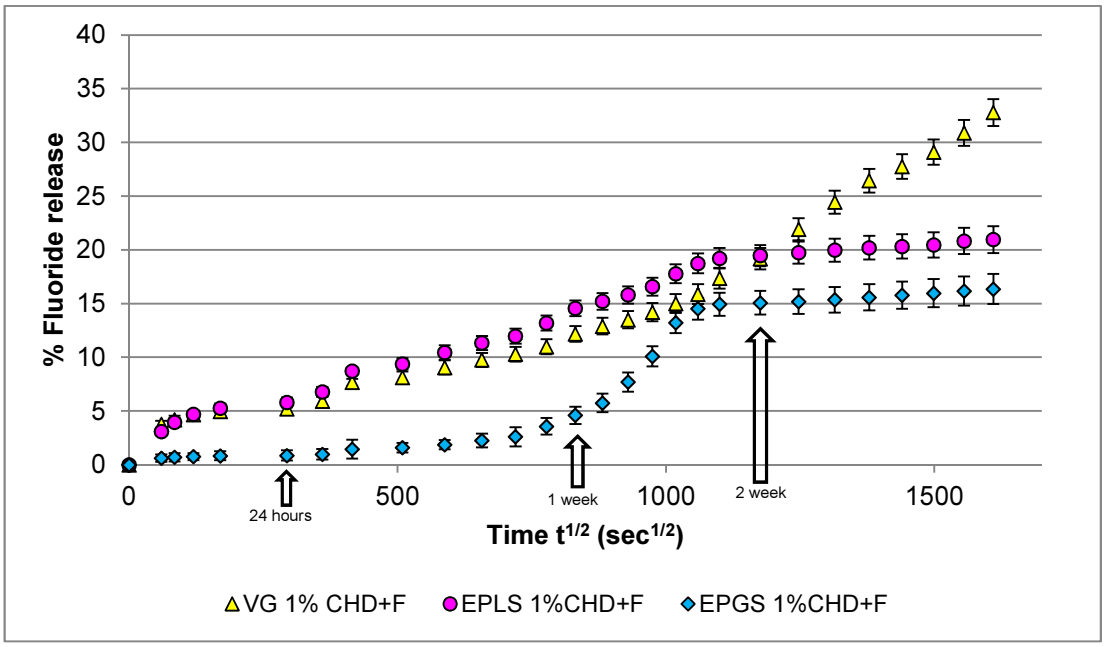

Figure 4.37: Mean (±SD; n=5) % fluoride release of 1% CHD formulations containing 0.5% NaF in DW at 37ºC for 4 weeks

VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

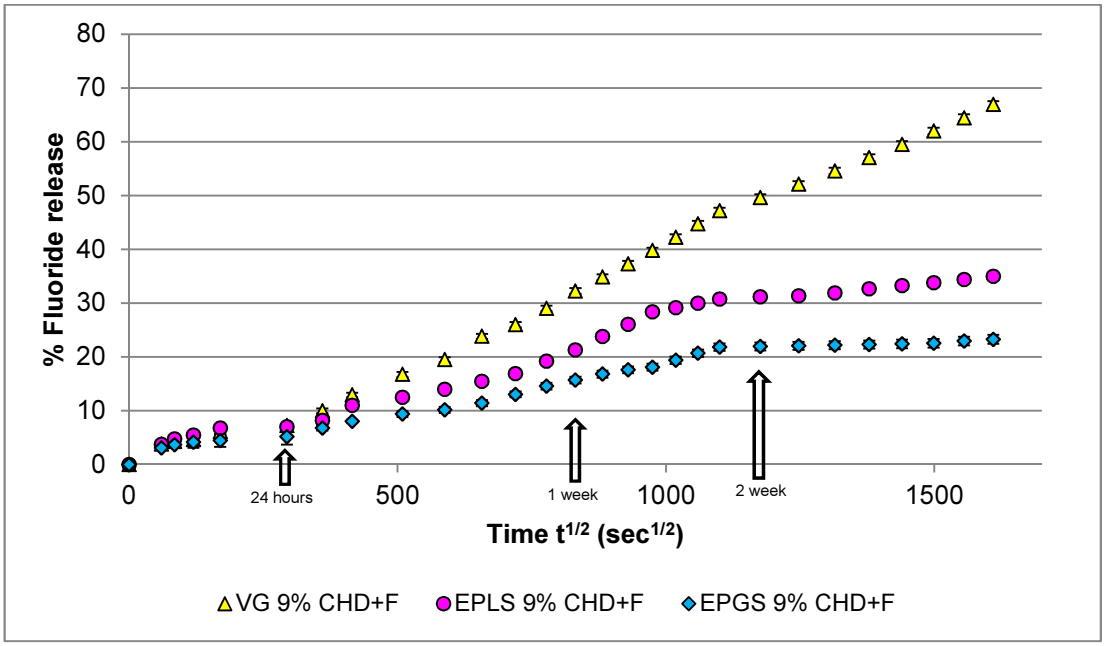

Figure 4.38: Mean (±SD; n=5) % fluoride release of 9% CHD formulations containing 0.5% NaF in DW at 37ºC for 4 weeks

VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

Table 4.8 summarizes the fluoride release as part per million (ppm) and % after 24 hours, 1 week and after 4 week. Here it is clearly seen that all 1% CHD formulations released less fluoride (both ppm and %) compared to their 9% CHD counterparts at all time points. At all time points, for both 1% and 9% CHD formulations EPGS released less F than VG and EPLS (in ppm and %).

At 1 day there was no significant difference in the F release of VG and EPLS for both levels of CHD. Although the EPGS formulations showed the lowest F released, increasing the CHD incorporated from 1% to 9% increased the amount of F released more than fivefold; this was higher than for VG and EPLS. After 1 week EPLS 1%CHD+F had released more F than either VG or EPGS, however for the 9%CHD formulations, VG had released the highest amount. Increasing the amount of CHD from 1% to 9% had more of an effect on release from EPGS and VG than EPLS.

After 4 weeks VG had released significantly more F than either EPLS or EPGS, particularly for the 9% CHD formulations. Differences in release may be related to ethanol content and solubility (see Table 4.4; page 156) where VG has by far the highest solubility.

Table 4.8: Summary of fluoride release at different time periods

Formulation	Fluoride release					
	1 Day		1 week		4 week	
	ppm	%	ppm	%	ppm	%
VG 1%CHD+F	0.61	5.2[a]	1.43	12.2	3.86	32.8
VG 9%CHD+F	0.85	7.3[b]	3.80	32.2	7.87	66.9
EPLS 1%CHD+F	0.73	5.8[a]	1.84	14.6[A]	2.65	21.0
EPLS 9%CHD+F	0.90	7.0[b]	2.72	21.3	4.46	34.9
EPGS 1%CHD+F	0.12	0.9	0.66	4.6	2.36	16.4
EPGS 9%CHD+F	0.71	5.1[a]	2.18	15.7[A]	3.23	23.3

(No significant difference between groups with same letters; $p \leq 0.05$)

From the graphs of %F release against $t^{1/2}$ of the different tissue conditioner formulations the gradients, after 24 hours and associated intercepts of the y-axis were calculated and shown in Table 4.9. The gradients of the plots show the rate of diffusion controlled release while the intercepts are an indication of the surface burst release. Negative values of the intercept on y-axis show absence of burst release in all 9%CHD+F formulations. EPGS formulations had the lowest release rates (gradients) while VG 9%CHD+F showed the highest.

Table 4.9: Gradients & intercepts of plots (%F release vs square root of time after 24 hours to 1 week) of % F release of tissue conditioner formulations containing 1% and 9% CHD with and without the addition of 0.5% NaF

Formulation	% F release gradients	% F release intercept
VG+1%CHD+F	0.0138	0.9243
VG+9%CHD+F	0.0492	-8.2664
EPLS+1%CHD+F	0.0162	1.0255
EPLS+9%CHD+F	0.0307	-3.224
EPGS+1%CHD+F	0.0029	0.2337
EPGS+9%CHD+F	0.02	-0.8912

4.9 Shore A Hardness and Young's Modulus

Low hardness is an essential requirement for tissue conditioners to function effectively; the required level will depend on intended application (Table 2.2; page 44). Shore A hardness values of all commercial and experimental materials were measured at 1 sec dwell time to minimize the effect of creep. The materials were stored at 37^{o}C in different storage media i.e. Dry, distilled water (DW) and artificial saliva (AS) before testing.

Effect of immersion on Shore A hardness varied between materials and storage conditions especially between dry and DW or AS. This reflects differences in gelation time, composition (e.g. ethanol content, which is highest in VG followed by EPLS and no ethanol in EPGS) and how the materials behave in the various environment (e.g. loss of ethanol, plasticiser etc).

4.9.1 Commercial and Experimental Materials

Two commercial materials CC and VG were selected for use as commercial controls as they are recommended for the main three clinical applications of tissue conditioners as described in section 2.3 (page 38). All the commercial materials were mixed using the manufacturer's recommended P/L ratio and an experimental P/L of 1.8 g/ml.

Figures 4.39 to 4.41 show the mean Shore A hardness of VG1.5 and 1.8, CC 1.2 and 1.8, EPLS and EPGS at different time periods when stored at 37°C in dry, DW and AS respectively. EPGS formulations were left to gel for 16 hours after mixing, so in all plots time of 1 hour is referred to as 16 hours after mixing. A similar pattern is seen for all formulations where the Shore A hardness increased with time in different media with the exception of dry (Figures 4.39 to 4.41). This increase in Shore A hardness was seen more in DW, followed by AS then dry. In CC and VG increasing the P/L ratio also increased the Shore A hardness.

When stored dry (Figure 4.39), Shore A hardness of all materials increased more over the first 24 hours and, then from 24 hours to 1 week, there was no significant difference except for CC 1.2. The increase in Shore A hardness from 1 hour to 24 hours reflects the continuation of the gelation process, which is more noticeable in the P/L formulations. The continuing increase of Shore A hardness from 24 hours to 1 week for CC 1.2 probably results from continuing gelation due to its lower P/L.

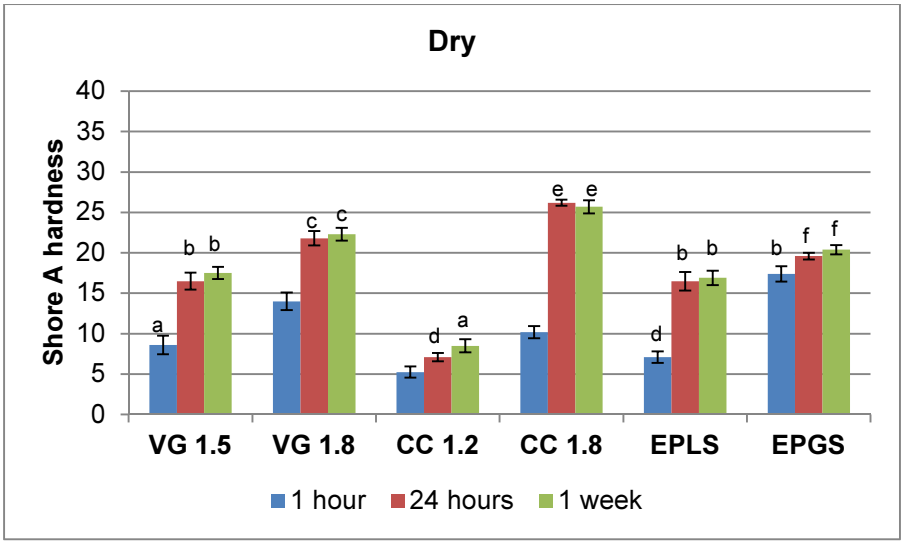

Figure 4.39: Mean (±SD; n=6) Shore A hardness values of CC and VG formulations at different P/L ratios, EPLS and EPGS stored dry at 37°C

(No significant difference between groups with same letters; p≤0.05)
VG 1.5: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.5g/ml
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
CC 1.2: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.2g/ml
CC 1.8: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

In DW (Figure 4.40) all formulations increased in Shore A hardness with time and with increasing P/L ratio for CC and VG. CC 1.2 showed a smaller increase in Shore A hardness from 1 hour to 24 hours and then a greater increase from 24 hours to 1 week, whereas with all other formulations the increase in Shore A hardness was more from 1 hour to 24 hours than from 24 hours to 1 week. The Shore A hardness for each of the materials were significantly different at 1 week, except for VG 1.5 and VG 1.8; EPLS and CC 1.2, where there was no statistical difference.

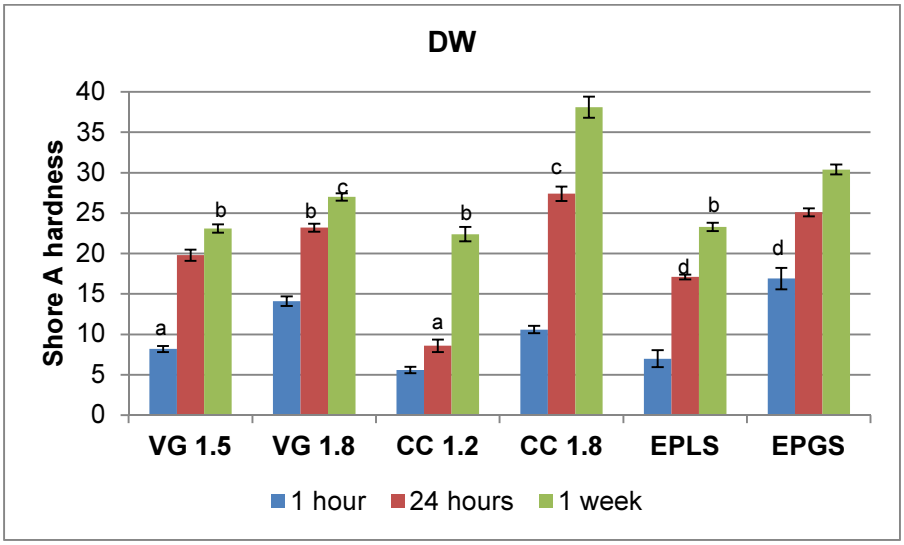

Figure 4.40 Mean (±SD; n=6) Shore A hardness values of CC and VG formulations at different P/L ratios, EPLS and EPGS stored in DW at 37°C

(No significant difference between groups with same letters; p≤0.05)

VG 1.5: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.5g/ml
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
CC 1.2: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.2g/ml
CC 1.8: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

In AS (Figure 4.41) again Shore A hardness increased with time in all formulations with the exception of VG 1.5 where there was no significant difference found between 24 hours and 1 week, indicating difference in the balance between the effect on Shore A hardness of plasticiser loss (hardening) and fluid uptake (softening). CC 1.2 had a greater increase from 24 hours to 1 week than 1 hour to 24 hours. At all time points the Shore A hardness of each of the materials were significantly different, except for VG 1.5 and EPLS at 1 week.

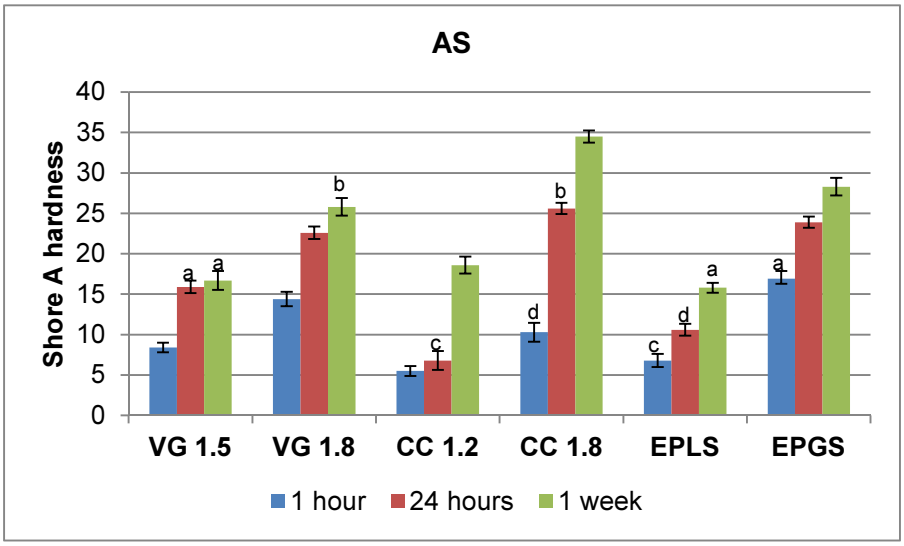

Figure 4.41 Mean (±SD; n=6) Shore A hardness values of CC and VG formulations at different P/L ratios, EPLS and EPGS stored in AS at 37°C

(No significant difference between groups with same letters; p≤0.05)

VG 1.5: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.5g/ml
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
CC 1.2: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.2g/ml
CC 1.8: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

Figure 4.42 shows the mean Young's modulus (calculated as explained in section 3.2.5; page 117) for CC 1.2 & 1.8, VG 1.5 & 1.8, EPLS at 24 hours and EPGS in dry. CC 1.8 showed the highest value of Young's modulus and CC 1.2 showed the lowest value. In CC and VG increasing the P/L ratio also increased the Young's modulus of the material. Note that the Young's modulus of EPGS (16 hours after mixing) is very similar to VG 1.5 and EPLS (no significant difference in Shore A hardness as shown in Figure 4.39 to Figure 4.41).

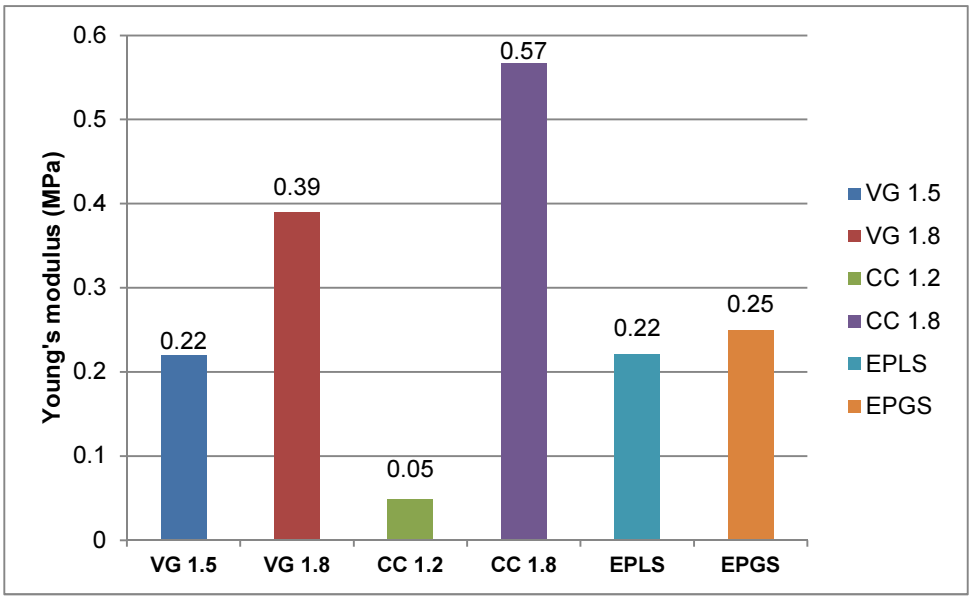

Figure 4.42: Mean (n=6) Young's modulus of CC, VG, EPLS formulations 24 hours after mixing and EPGS

VG 1.5: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.5g/ml
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
CC 1.2: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.2g/ml
CC 1.8: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

4.9.2 Addition of CHD with and without 0.5% NaF

This section of the results shows the effect of the addition of 1% or 9% CHD with and without 0.5% NaF, on the Shore A hardness and Young's modulus of VG 1.8, EPLS and EPGS formulations. The results are displayed so that the effect of increase in CHD from 1% to 9%, and the effect of the addition of 0.5% NaF, can be compared in different storage media. Changes in Shore A hardness as well as being influenced by the factors noted at the start of this section will additionally be affected by the presence of additives.

Figures 4.46 to 4.52 show the mean Shore A hardness of VG 1.8, EPLS and EPGS 1% or 9% CHD formulations, with and without the addition of 0.5% NaF, stored at 37°C in dry, DW and AS respectively. Generally, the Shore A hardness values increased with increasing time in all media, the increase being highest in DW followed by AS and then dry. When stored dry, there was little or no change in Shore A hardness between 24 hours and 1 week with some exceptions which are highlighted below.

Figures 4.43 to 4.45 show the mean Shore A hardness of VG 1.8, 1% or 9% CHD formulations with and without the addition of 0.5% NaF, stored at 37°C in dry, DW and AS respectively. At 1 hour the addition of 1% CHD increased the Shore A hardness but there was no significant difference found when NaF was added. Increasing CHD from 1% to 9% decreased the Shore A hardness and the addition of NaF to 9%CHD increased the Shore A hardness significantly at 1 hour. Increase in Shore A hardness from 1 hour to 24 hours was more in 9%CHD than other

formulations. For VG stored dry (Figure 4.43), (except for 1%CHD), there was no significance difference found between Shore A hardness at 24 hours and 1 week.

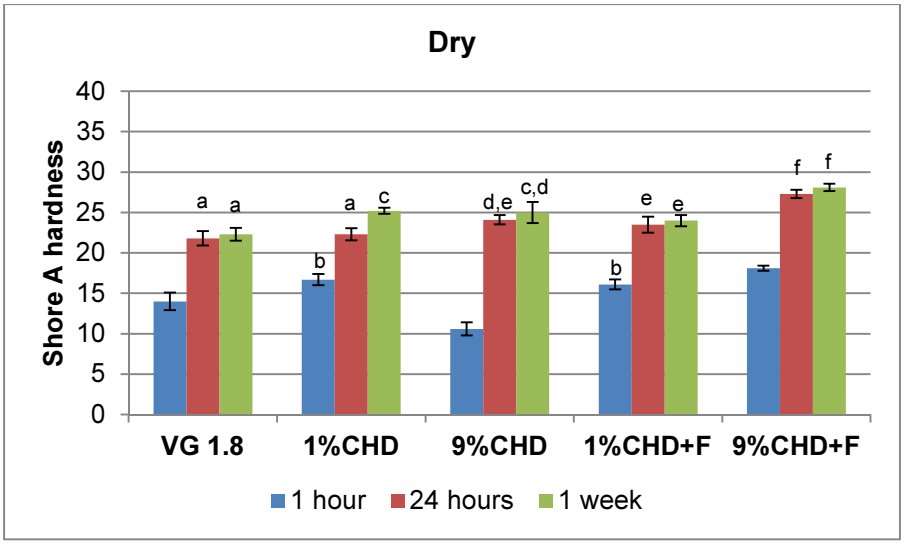

Figure 4.43: Mean (±SD; n=6) Shore A hardness values of VG, VG 1% and 9% CHD with and without 0.5% NaF stored dry at 37°C

(No significant difference between groups with same letters; p≤0.05)
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml

In DW (Figure 4.44) although the Shore A hardness of VG 1.8 increased with the addition of 1%CHD it was lower at 24 hours and, at 1week, there was no significant difference between the two. Increasing the CHD from 1% to 9%CHD decreased Shore A hardness at 1 hour but at 24 hours and 1 week the values were higher. Addition of NaF to 9%CHD increased the Shore A hardness which were significantly higher at each time point. A similar trend was also seen in AS (Figure 4.45).

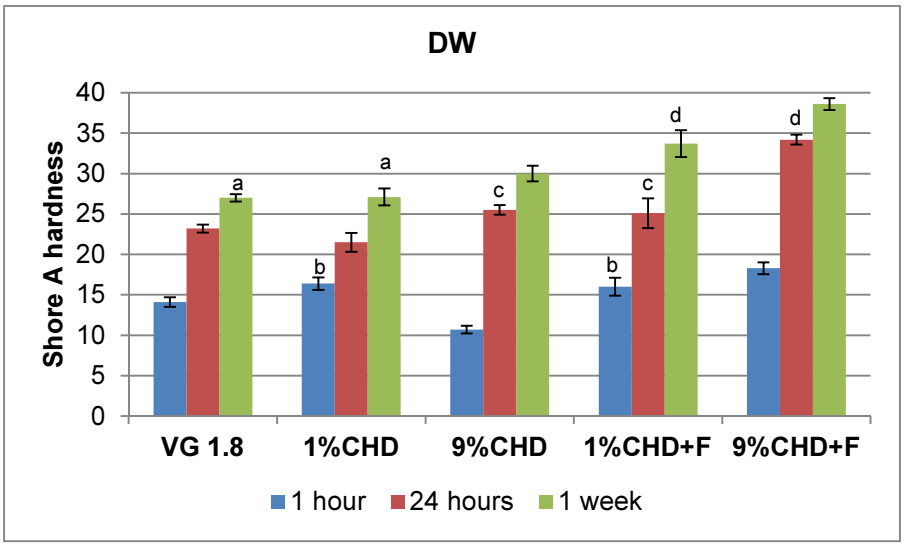

Figure 4.44 Mean (±SD; n=6) Shore A hardness values of VG, VG 1% and 9% CHD with and without 0.5% NaF stored in DW at 37°C

(No significant difference between groups with same letters; p≤0.05)
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml

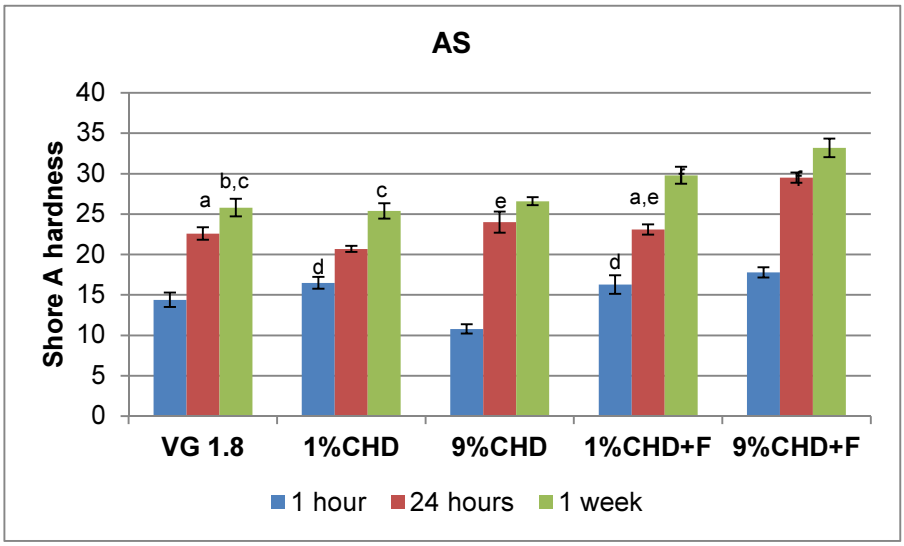

Figure 4.45 Mean (±SD; n=6) Shore A hardness values of VG, VG 1% and 9% CHD with and without 0.5% NaF stored in AS at 37°C

(No significant difference between groups with same letters; p≤0.05)
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml

Figures 4.46 to 4.48 show the mean Shore A hardness of EPLS, 1% or 9% CHD formulations with and without the addition of 0.5% NaF, stored at 37°C in dry, DW and AS respectively. Addition of 1%CHD to EPLS resulted in an increase in Shore A hardness which was further increased in the 9%CHD specimens. Addition of NaF to both 1% and 9%CHD had no significant effect on the Shore A hardness at 1 hour. In all storage conditions addition of NaF to 1%CHD resulted in a further increase at 1 week but this effect was not seen for 9%CHD.

Stored dry (Figure 4.46), the Shore A hardness of EPLS increased significantly from 24 hours to 1 week for 9%CHD+F; for all other formulations there was no significant difference between values after 24 hours and 1 week.

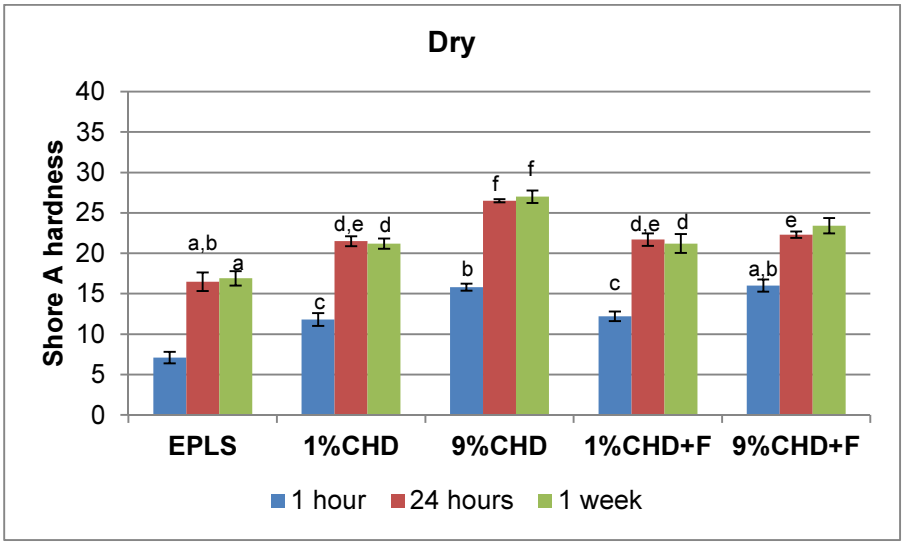

Figure 4.46: Mean (±SD; n=6) Shore A hardness values of EPLS 1% and 9% CHD with and without 0.5% NaF stored dry at 37°C

(No significant difference between groups with same letters; p≤0.05)
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml

In DW (Figure 4.47) the Shore A hardness of EPLS showed no significant difference between 1%CHD and 1%CHD+F after 24 hours and between 1%CHD+F and 9%CHD+F at both 24 hours and 1 week.

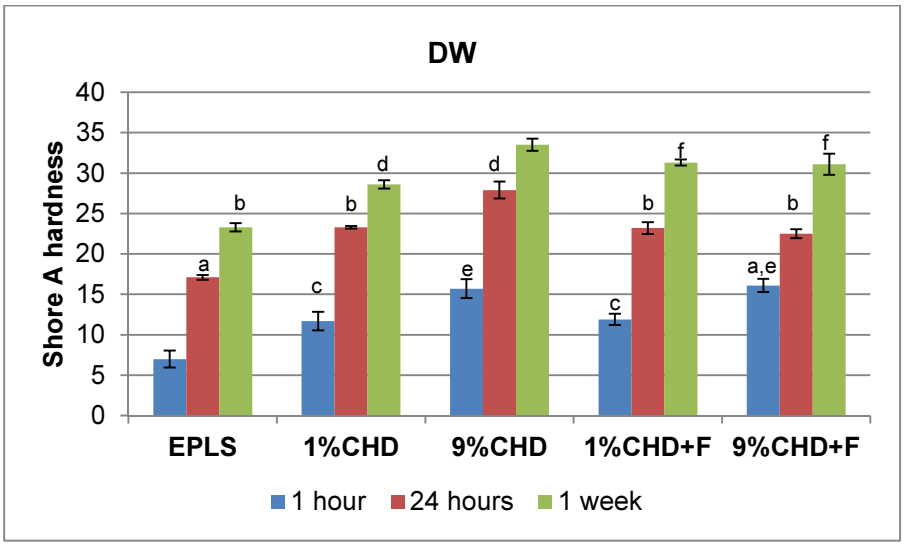

Figure 4.47 Mean (±SD; n=6) Shore A hardness values of EPLS 1% and 9% CHD with and without 0.5% NaF stored in DW at 37°C

(No significant difference between groups with same letters; p≤0.05)
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml

In AS (Figure 4.48), addition of 1% CHD increased the Shore A hardness at all time points, which was further increased when CHD was increased to 9%. Addition of NaF increased the Shore A hardness in 1%CHD after 24 hours and 1week but reduced the Shore A hardness of 9%CHD at the same time points.

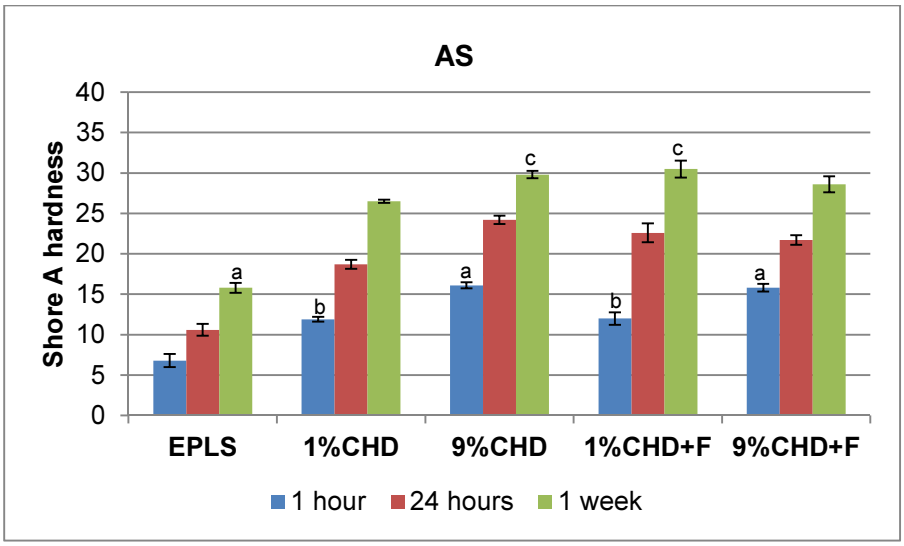

Figure 4.48 Mean (±SD; n=6) Shore A hardness values of EPLS 1% and 9% CHD with and without 0.5% NaF stored in AS at 37°C

(No significant difference between groups with same letters; p≤0.05)
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml

Figures 4.49 to 4.51 show the mean Shore A hardness of EPGS, 1% and 9% CHD formulations with and without addition of 0.5% NaF stored at 37°C dry, in DW and in AS respectively. In all media, at 1 hour the Shore A hardness of EPGS increased when 1%CHD was added but no significant difference was found when NaF was added to 1%CHD. Also Shore A hardness decreased when CHD was increased from 1% to 9% at the same time points.

Stored dry (Figure 4.49), all formulations of EPGS increased in Shore A hardness from 24 hours to 1 week except EPGS and 9%CHD+F. There was also no significant difference between 1% and 9% CHD after 1 week.

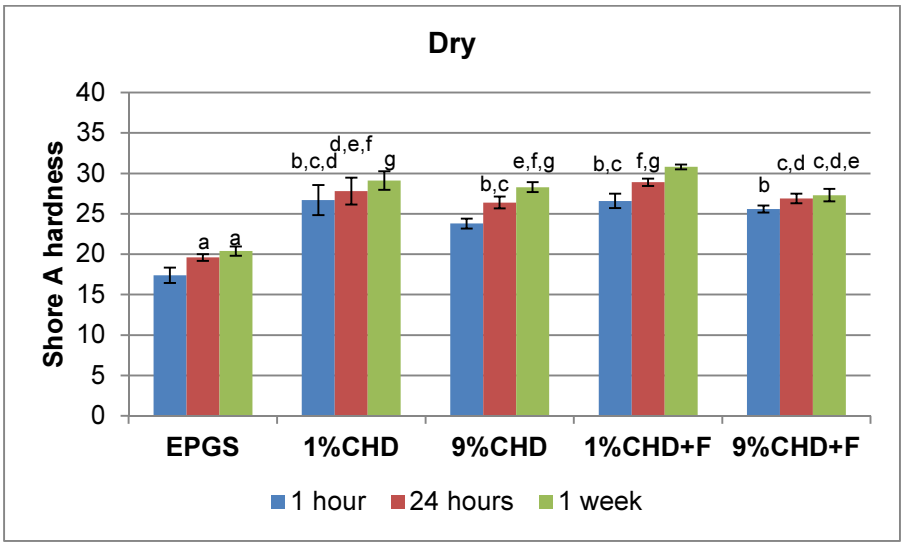

Figure 4.49: Mean (±SD; n=6) Shore A hardness values of EPGS 1% and 9% CHD with and without 0.5% NaF stored dry at 37°C

(No significant difference between groups with same letters; p≤0.05)
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

In DW (Figure 4.50), Shore A hardness of EPGS showed no significant difference in 1% and 9%CHD with and without NaF after 24 hours. However, Shore A hardness increased after 1 week when 1%CHD was added and further increased when CHD was increased to 9%, but addition of NaF decreased the Shore A hardness significantly for both.

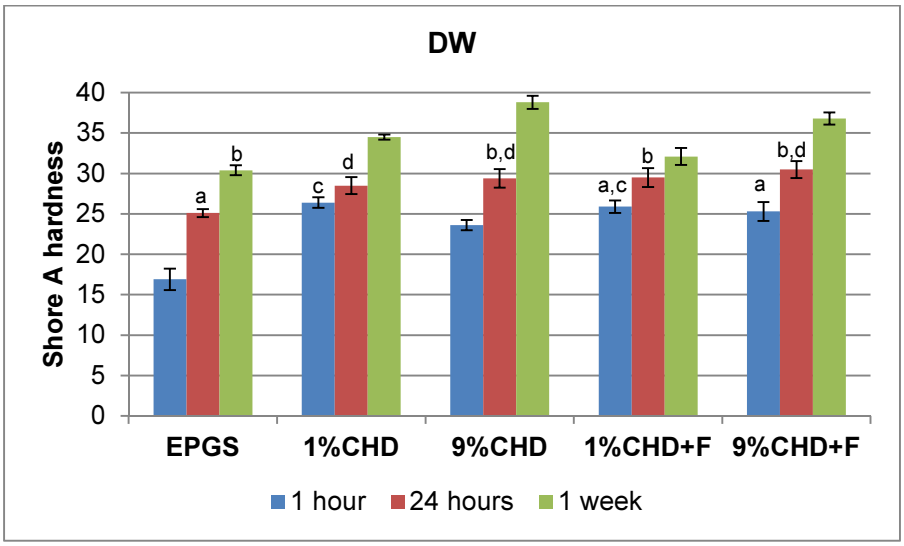

Figure 4.50: Mean (±SD; n=6) Shore A hardness values of EPGS 1% and 9% CHD with and without 0.5% NaF stored in DW at 37°C

(No significant difference between groups with same letters; p≤0.05)
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

Figure 4.51 showed that after 24 hours EPGS 9%CHD had a higher Shore A hardness than 1%CHD but when NaF was added there was no significant difference between the two. Also Shore A hardness showed a similar trend in AS after 1 week as seen in DW, but to a lesser extent.

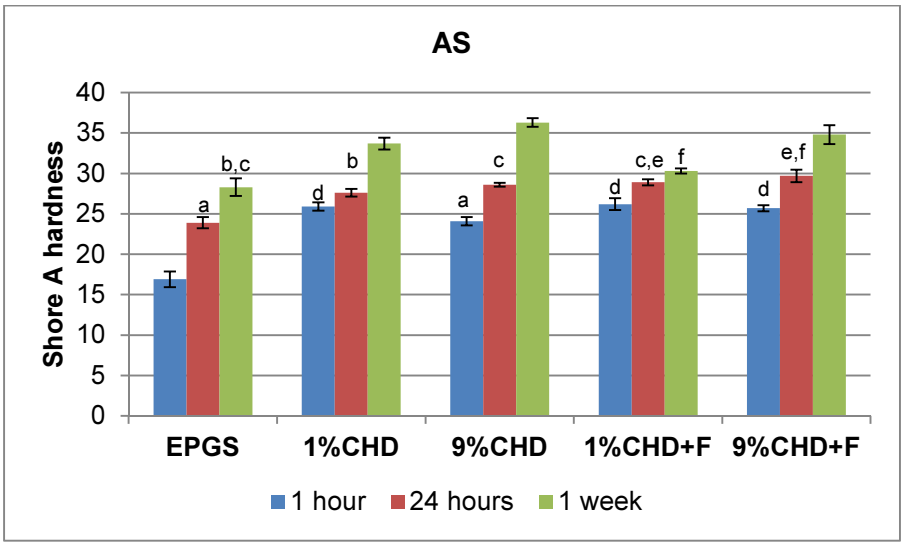

Figure 4.51: Mean (±SD; n=6) Shore A hardness values of EPGS 1% and 9% CHD with and without 0.5% NaF stored in AS at 37°C

(No significant difference between groups with same letters; p≤0.05)
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

Figure 4.52 shows a comparison of the mean Shore A hardness of VG 1.8, EPLS after 24 hours and EPGS with and without additives. Addition of CHD with or without NaF had more effect on EPLS and EPGS compared to VG 1.8. Effect of additives varies between materials and is probably related to differences in composition, particularly ethanol content. Comparing between materials, with no additives VG 1.8 had the highest Shore A hardness with no significant difference between EPLS and

EPGS. The addition of 1%CHD increased Shore A hardness except for VG1.8, VG 1%CHD and EPLS 1%CHD having similar Shore A hardness. Increasing CHD to 9% increased Shore A hardness for VG and EPLS but decreased Shore A hardness for EPGS, where there was no significant difference between VG 9%CHD and EPGS 9%CHD. For VG and EPGS adding NaF increased Shore A hardness for 1% and 9%CHD, but for EPLS there was no increase in 1%CHD and a decrease for 9%CHD.

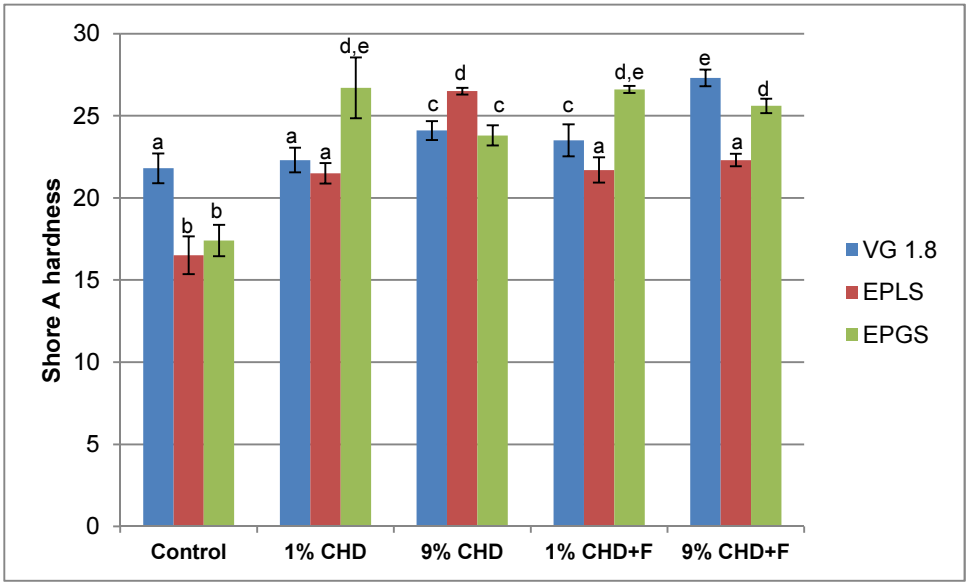

Figure 4.52: Mean (±SD; n=6) Shore A hardness values of VG, EPLS (24 hour after mixing) and EPGS, 1% and 9% CHD with and without 0.5% NaF formulations in dry

(No significant difference between groups with same letters; p≤0.05)
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

Figure 4.53 shows the mean Young's modulus of VG, EPLS and EPGS formulations with and without the addition of the additives. The trends seen here are similar (as would be expected) to the ones seen in Figure 4.52. It should be noted that Young's modulus was calculated for each materials at each time point, under each condition, and these are displayed in the Appendix.

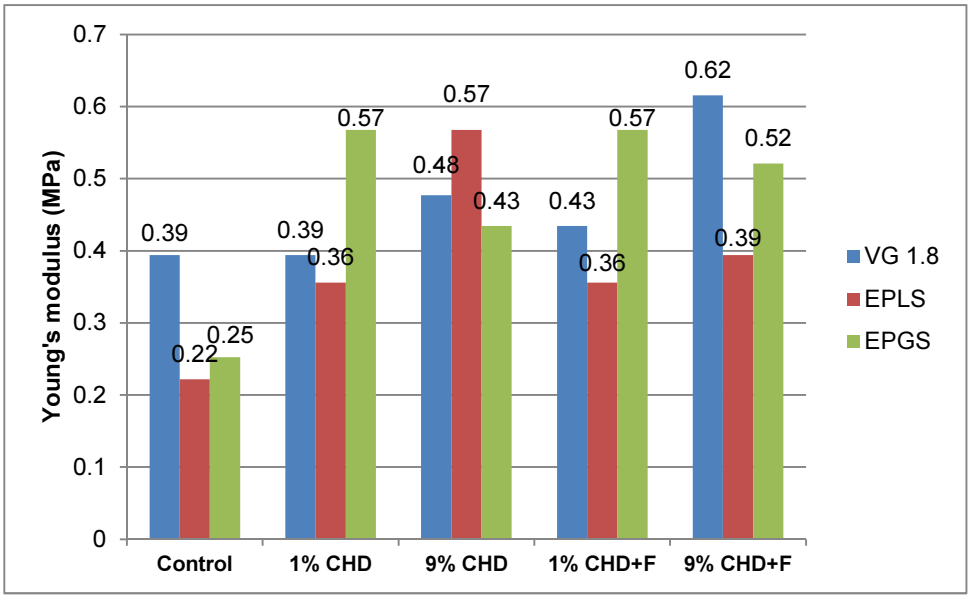

Figure 4.53: Mean Young's modulus of VG, EPLS (24 hour after mixing) and EPGS, 1% and 9% CHD with and without 0.5% NaF formulations in dry oven

VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

4.10 Creep Compliance Ratio

Creep compliance ratio (CCR) was calculated from Shore A hardness, where the materials were tested using 1, 5, 10, 15, 20, 25, and 30 sec dwell times as described in Section 3.2.6 (page 117). Flow properties of the materials are important

in determining suitability for tissue conditioner applications as detailed in section 2.6.3 (page 68). Here creep is used to measure the flow. The higher the creep in a material the greater will be the flow.

4.10.1 Commercial and Experimental Materials

The Shore A hardness of the different formulations decreased with increasing dwell time and this is seen in all the formulations at different time periods when stored in different media but to different extents. A typical plot of Shore A hardness at different dwell times is shown in Figure 4.54 which shows the results for CC1.2, CC 1.8, VG 1.5, VG 1.8, EPLS and EPGS formulations stored dry for 24 hours at 37°C. The rest of the Shore A hardness data at 1 hour, 24 hours and 1 week when stored dry, DW and in AS with and without additives are given in the Appendix.

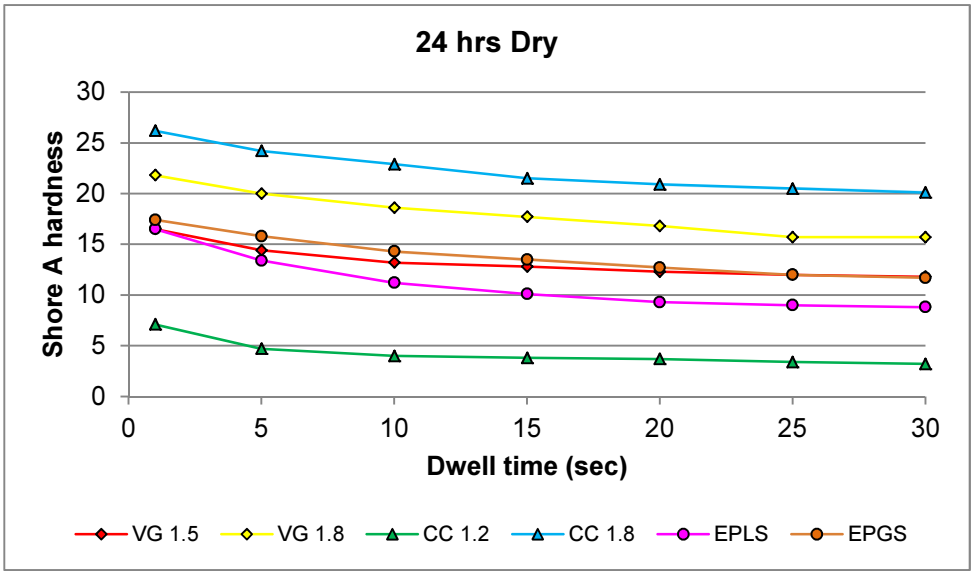

Figure 4.54: Mean (n=6) Shore A hardness values of VG, CC, EPLS and EPGS formulations at different dwell times stored at 37°C dry for 24 hours

VG 1.5: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.5g/ml
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
CC 1.2: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.2g/ml
CC 1.8: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

Table 4.10 shows the mean CCR of CC (1.5 and 1.8), VG (1.5 and 1.8) and EPLS at 1 hour and EPGS using a 30 sec dwell time for maximum effect of creep. EPLS showed the highest CCR followed by VG 1.8 whereas EPGS had the lowest CCR compared to all other formulations. A high CCR indicates more flow (creep) in the material.

Table 4.10: Mean (±SD; n=6) CCR of different formulations using 30 sec dwell time after 1 hour

Material	CCR after 1 hour
CC 1.2	6.3 ± 0.4
CC 1.8	5.1 ± 0.04
VG 1.5	64.0 ± 6.4
VG 1.8	31.4 ± 1.3
EPLS	49.0 ± 5.5
EPGS	2.6 ± 0.1

Figures 4.55 to 4.60 shows the mean CCR of CC (1.2 and 1.8), VG (1.5 and 1.8) EPLS and EPGS formulations when stored at 37°C for a) 24 hours and b) 1week dry, in DW and in AS respectively. The CCR in general increased with increasing dwell time and decreased with the storage time, with a few exceptions which are discussed below. Interaction with the immersion fluids will effect CCR as well as differences in the materials composition.

Figures 4.55 and 4.56 show the mean CCR of CC (1.2 and 1.8), VG (1.5 and 1.8) EPLS and EPGS formulations when stored at 37°C dry after 24 hours and 1 week

respectively. The CCR of all the formulations showed an increase from 24 hours to 1 week except CC1.8 and VG1.8. This increase was more in CC1.2 than the other formulations (Table 4.11) which could be attributed to its low P/L ratio.

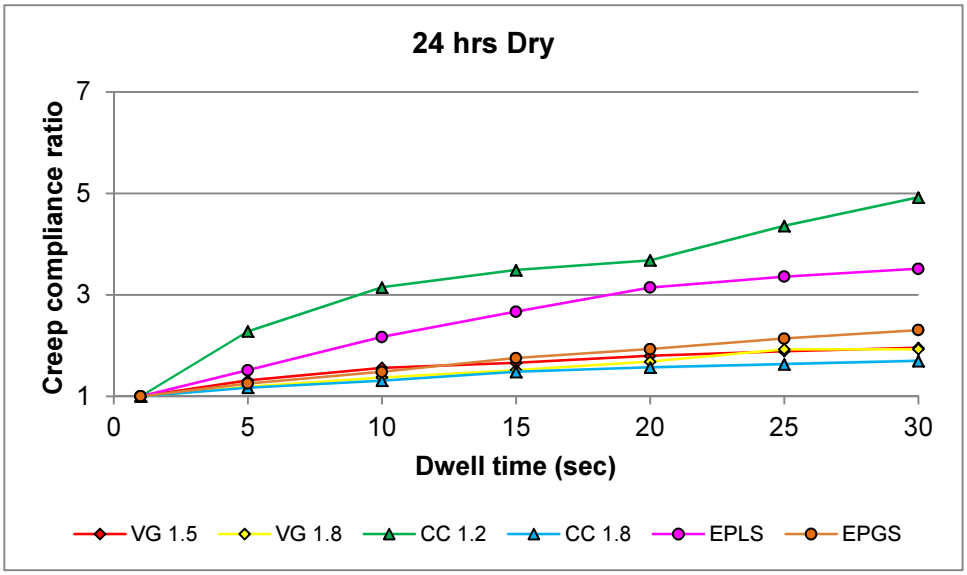

Figure 4.55: Mean CCR of VG, CC, EPLS and EPGS at different dwell times when stored at 37°C in dry oven after 24 hours

VG 1.5: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.5g/ml
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
CC 1.2: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.2g/ml
CC 1.8: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

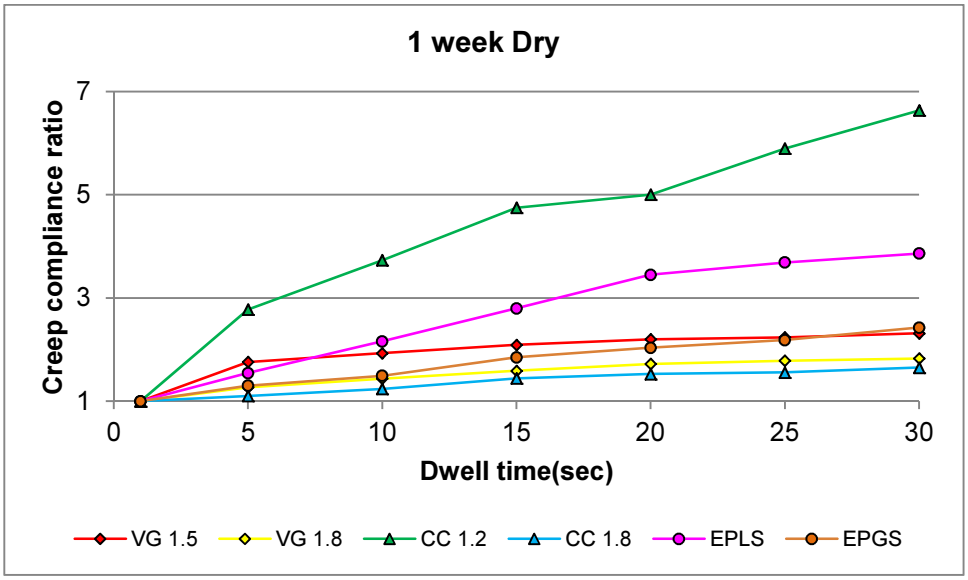

Figure 4.56: Mean CCR of VG, CC, EPLS and EPGS at different dwell times when stored at 37°C in dry oven after 1 week

VG 1.5: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.5g/ml
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
CC 1.2: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.2g/ml
CC 1.8: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

Figures 4.57 and 4.58 show the mean CCR of VG CC (1.5 and 1.8), VG (1.5 and 1.8) EPLS and EPGS formulations when stored at 37°C in DW after 24 hours and 1 week respectively. All formulations showed a reduction in CCR with time but to a different extent. EPLS showed the highest CCR, both after 24 hours and 1 week, and VG 1.8 showed the least change in CCR.

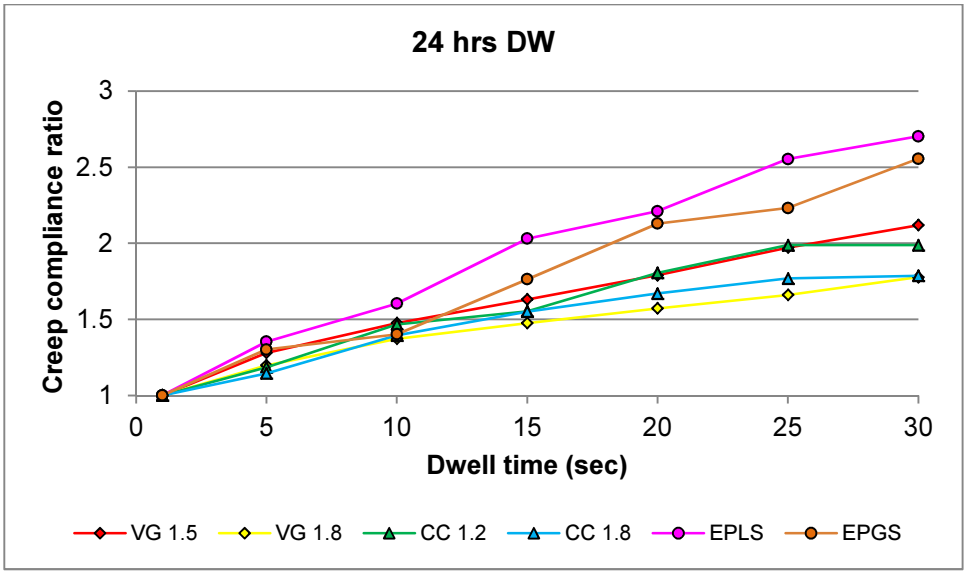

Figure 4.57: Mean CCR of VG, CC, EPLS and EPGS at different dwell times when stored at 37°C in DW after 24 hours

VG 1.5: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.5g/ml
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
CC 1.2: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.2g/ml
CC 1.8: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

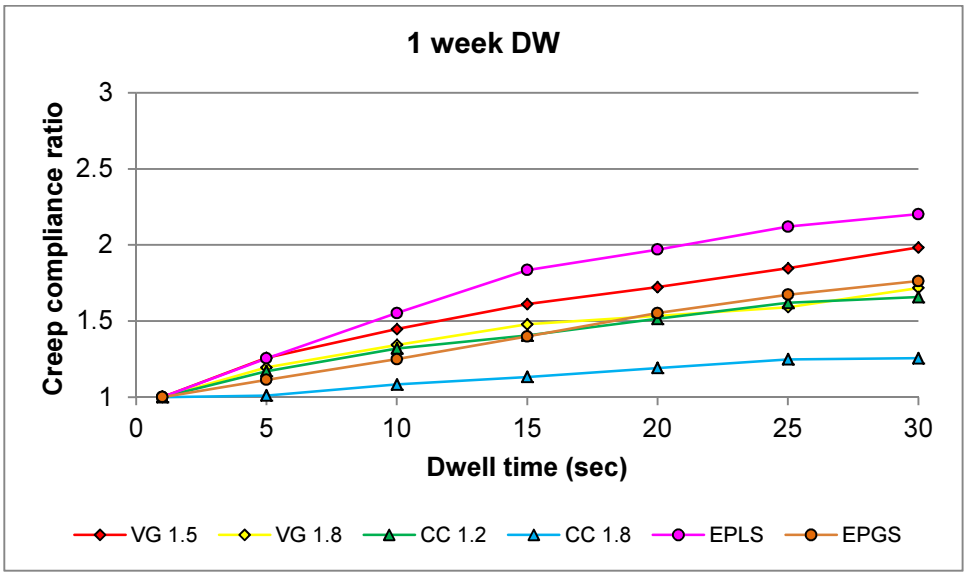

Figure 4.58: Mean CCR of VG, CC, EPLS and EPGS at different dwell times when stored at 37°C in DW after 1 week

VG 1.5: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.5g/ml
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
CC 1.2: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.2g/ml
CC 1.8: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

Figures 4.59 and 4.60 show the mean CCR of VG CC (1.5 and 1.8), VG (1.5 and 1.8) EPLS and EPGS formulations when stored at 37°C in AS after 24 hours and 1 week respectively. EPLS and CC 1.2 showed the highest CCR after 24 hours whereas after 1 week VG 1.5 and EPLS showed the highest CCR. CCR generally decreased from 24 hrs to 1 week, but there was little or no change for CC1.8, VG 1.5 and VG 1.8.

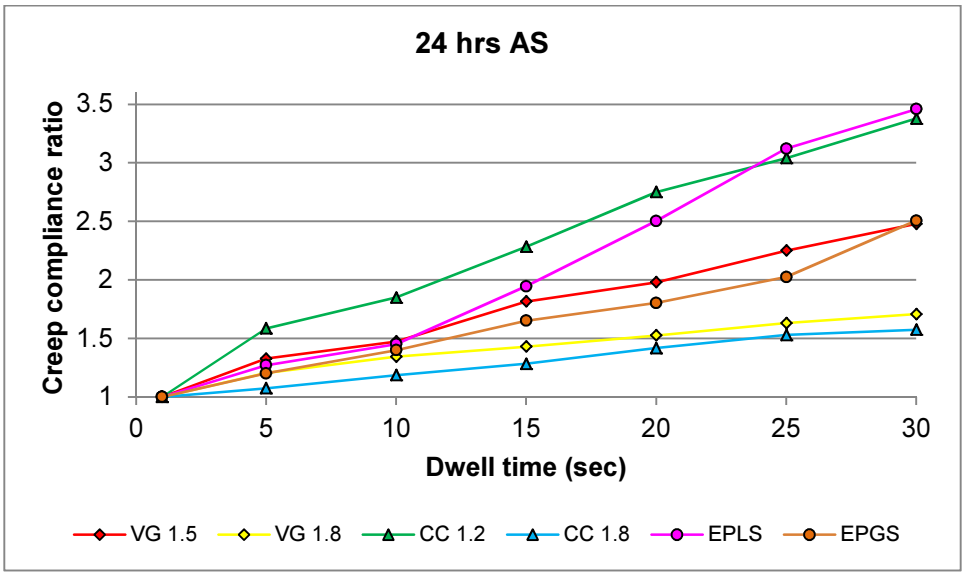

Figure 4.59: Mean CCR of VG, CC, EPLS and EPGS at different dwell times when stored at 37°C in AS after 24 hours

VG 1.5: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.5g/ml
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
CC 1.2: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.2g/ml
CC 1.8: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

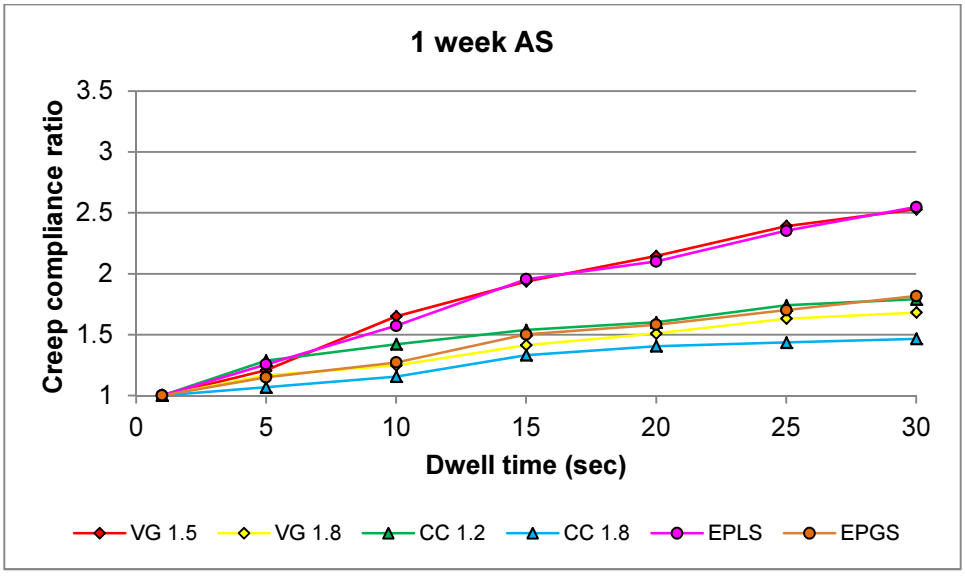

Figure 4.60: Mean CCR of VG, CC, EPLS and EPGS at different dwell times when stored at 37°C in AS after 1week

VG 1.5: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.5g/ml
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml
CC 1.2: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.2g/ml
CC 1.8: PEMA powder & benzyl benzoate+ ester stearic acid+6.2% ethanol; P/L ratio 1.8g/ml
EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

Table 4.11 summarises the mean CCR of different formulations using 30 sec dwell time after 24 hours and 1 week dry, in DW and in AS. Stored dry, CCR either increased or remained constant with time, whereas in DW and AS CCR either decreased or remained constant. When immersed in either DW or AS CC 1.2, EPLS and EPGS showed higher decreases after 1 week.

Table 4.11: Mean (±SD; n=6) CCR of different formulations using 30 sec dwell time after 24 hours and 1 week

Material	Dry		DW		AS	
	24 hour	1 week	24 hour	1 week	24 hour	1 week
CC 1.2	4.9 ± 0.05	6.6 ± 0.03	2.0^{A} ± 0.01	1.7^{B} ± 0.07	3.4^{-} ± 0.06	1.8^{*} ± 0.04
CC 1.8	1.7^{a} ± 0.01	1.7^{a} ± 0.03	1.8^{C} ± 0.06	1.3 ± 0.03	1.6^{*} ± 0.04	1.5^{*} ± 0.02
VG 1.5	2.0^{b} ± 0.2	2.3^{c} ± 0.04	2.1^{D} ± 0.04	2.0^{A} ± 0.01	2.5^{+} ± 0.03	2.5^{+} ± 0.17
VG 1.8	1.9^{b} ± 0.02	$1.8^{a,b}$ ± 0.04	1.8^{C} ± 0.01	$1.7^{B,C}$ ± 0.02	1.7^{*} ± 0.002	1.7^{*} ± 0.17
EPLS	3.5 ± 0.2	3.9 ± 0.09	2.7 ± 0.01	2.2^{D} ± 0.01	3.5^{-} ± 0.1	2.5^{+} ± 0.02
EPGS	2.3^{c} ± 0.1	2.4^{c} ± 0.02	2.6 ± 0.04	1.8^{C} ± 0.01	2.5^{+} ± 0.01	1.8^{*} ± 0.06

(No significant difference between groups with same letters/symbols; p≤0.05)

Table 4.12 shows the penetration ratio of different formulations of tissue conditioners after 24 hours and 1 week dry, in DW and in AS. When stored dry penetration ratio generally increased in all formulations except VG where it decreased particularly VG 1.5. When stored in DW and AS the ratio decreased in all formulations with the exception of VG when stored in AS where the ratio increased, particularly VG 1.5 and in DW where there was no change.

Table 4.12: Penetration ratio (R) of different formulations after 24 hours and 1 week

Material	Dry		DW		AS	
	24 hour	1 week	24 hour	1 week	24 hour	1 week
CC 1.2	1.47	1.55	1.3	1.19	1.46	1.18
CC 1.8	1.2	1.23	1.25	1.11	1.21	1.17
VG 1.5	1.42	1.15	1.26	1.26	1.37	1.45
VG 1.8	1.27	1.2	1.22	1.2	1.19	1.2
EPLS	1.52	1.58	1.41	1.32	1.65	1.42
EPGS	1.36	1.37	1.4	1.26	1.44	1.26

4.10.2 Addition of CHD with and without 0.5% NaF

Table 4.13 shows the mean CCR of VG, EPLS and EPGS, 1% and 9% CHD formulations with and without the addition of 0.5% NaF at 30 sec dwell time. It should be noted that the CCR of VG and EPLS are at 1 hour after mixing whereas EPGS takes 16 hour to gel and the readings are after I hour i.e. 17 hours after mixing. EPLS formulations showed the highest CCR followed by VG formulations and EPGS showed the lowest CCR. Addition of 1% CHD to the controls decreased the CCR. Increasing the CHD from 1% to 9% increased the CCR and addition of NaF had a variable effect, increasing the CCR for VG 1%CHD and EPGS but decreasing for VG 9%CHD and EPLS formulations. EPGS had the lowest CCR but this was the least affected by the addition of CHD or NaF. CCR were also calculated for 5, 10, 15, 20 and 25 sec dwell time for each material when stored dry, in DW and in AS, 1 hour, 24 hours and 1 week after mixing. These data follow the trends in Table 4.13 and Figures 4.61 to 4.69, and are displayed in the Appendix.

Table 4.13: Mean (±SD; n=6) CCR of VG and EPLS after 1 hour and EPGS; 1 & 9% CHD with or without 0.5% NaF using 30 sec dwell time

Material	1 hour
VG 1.8	31.4 ± 1.3
VG 1%CHD	6.8 ± 0.2
VG 9%CHD	23.2 ± 1.8
VG 1%CHD+F	10.0 ± 0.3
VG 9%CHD+F	9.1 ± 0.4
EPLS	49.0 ± 5.5
EPLS 1%CHD	16.6 ± 3.2
EPLS 9%CHD	26.0 ± 4.8
EPLS 1%CHD+F	10.3 ± 0.3
EPLS 9%CHD+F	15.2 ± 0.6
EPGS	2.6 ± 0.1
EPGS 1%CHD	2.3 ± 0.1
EPGS 9%CHD	2.6 ± 0.02
EPGS 1%CHD+F	2.9 ± 0.05
EPGS 9%CHD+F	2.7 ± 0.1

Figures 4.61 to 4.63 show the mean CCR of VG 1.8, VG 1% and 9% CHD, with and without 0.5% NaF using 30 sec dwell time after 24 hours and 1 week when stored dry, in DW and in AS respectively at 37°C.

Dry (Figure 4.61), mean CCR values of VG 1.8 formulations decreased from 24 hours to 1 week but for all except 9%CHD+F there was no statistical difference, also there were no statistical difference in CCR between 24 hours and 1 week for VG 1.8 and VG 1%CHD+F formulations. Addition of NaF to 1%CHD increased CCR at both time points. Addition of 9%CHD increased the CCR at both time points, which was further increased when NaF was added.

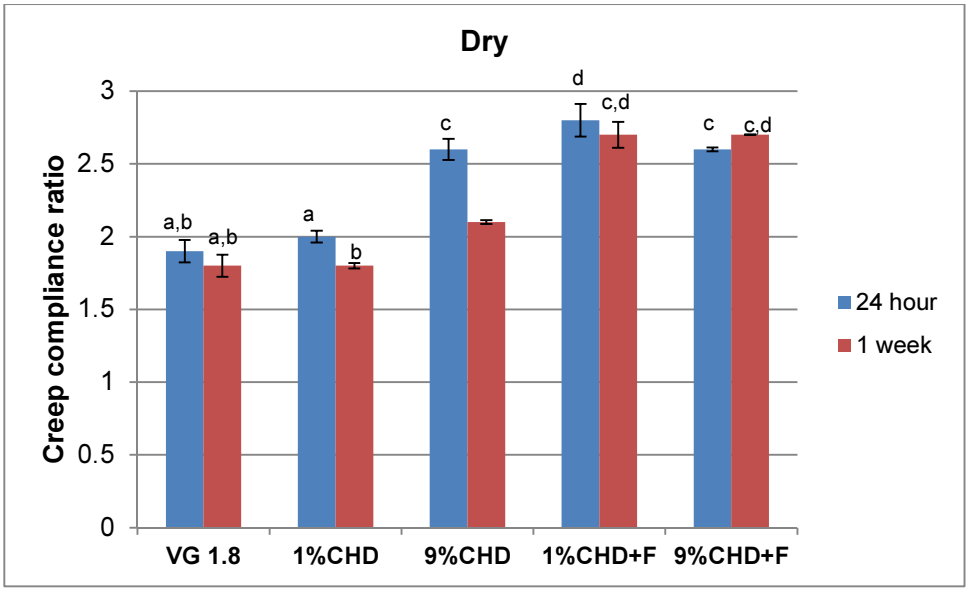

Figure 4.61: Mean (±SD; n=6) CCR of VG, VG 1% and 9% CHD with and without 0.5% NaF using 30 sec dwell time stored dry at 37°C

(No significant difference between groups with same letters; p≤0.05)

VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml

In DW (Figure 4.62), there was no effect of immersion time on CCR of VG 1.8 formulations except for 9%CHD, where it increased and 9 %CHD+F, where it decreased. At both time points addition of 1%CHD increased CCR which was further increased in 9% CHD. Addition of NaF had no effect on CCR for 1% CHD but decreased it for 9% CHD.

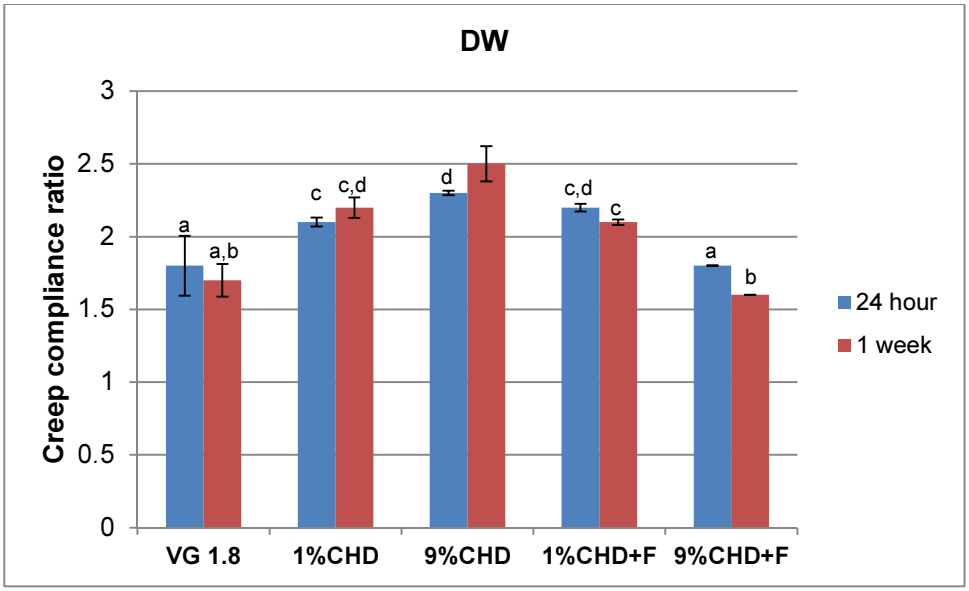

Figure 4.62: Mean (±SD; n=6) CCR of VG, VG 1% and 9% CHD with and without 0.5% NaF using 30 sec dwell time stored in DW at 37°C

(No significant difference between groups with same letters; p≤0.05)
VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml

In AS (Figure 4.63), there was no significance difference in CCR after 24 hours and 1 week for VG 1.8, 9%CHD and 1%CHD+F whereas it decreased in the other formulations with time from 24 hours to 1 week. Addition of 1% and 9% CHD increased the CCR at both time points which was further increased when NaF was added to 1%CHD (no significant difference at 24 hours) but decreased for 9%CHD.

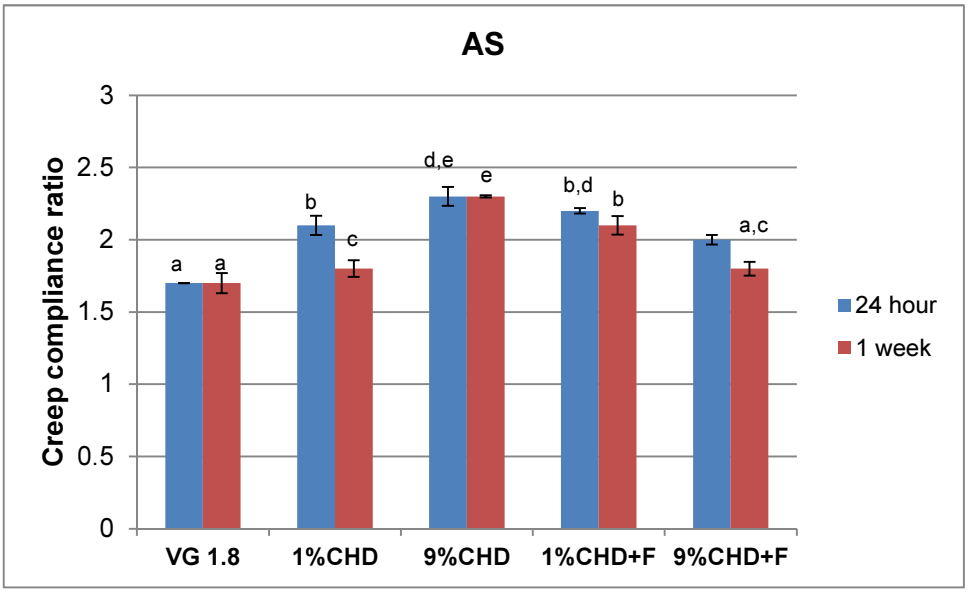

Figure 4.63: Mean (±SD; n=6) CCR of VG, VG 1% and 9% CHD with and without 0.5% NaF using 30 sec dwell time stored in AS at 37°C

(No significant difference between groups with same letters; p≤0.05)

VG 1.8: PEMA powder & 90%ATBC+10%ethanol; P/L ratio 1.8g/ml

Figures 4.64 to 4.66 shows the mean CCR of EPLS, EPLS 1% and 9% CHD, with and without 0.5% NaF using 30 sec dwell time at 24 hours and 1 week when stored dry, in DW and in AS respectively at 37°C.

Dry (Figure 4.64), there was no statistical difference in CCR of EPLS after 24 hours and 1 week for all formulations. Addition of 1%CHD increased the CCR but was decreased when CHD was increased to 9% and further decreased when NaF was added to both 1% and 9% CHD formulations.

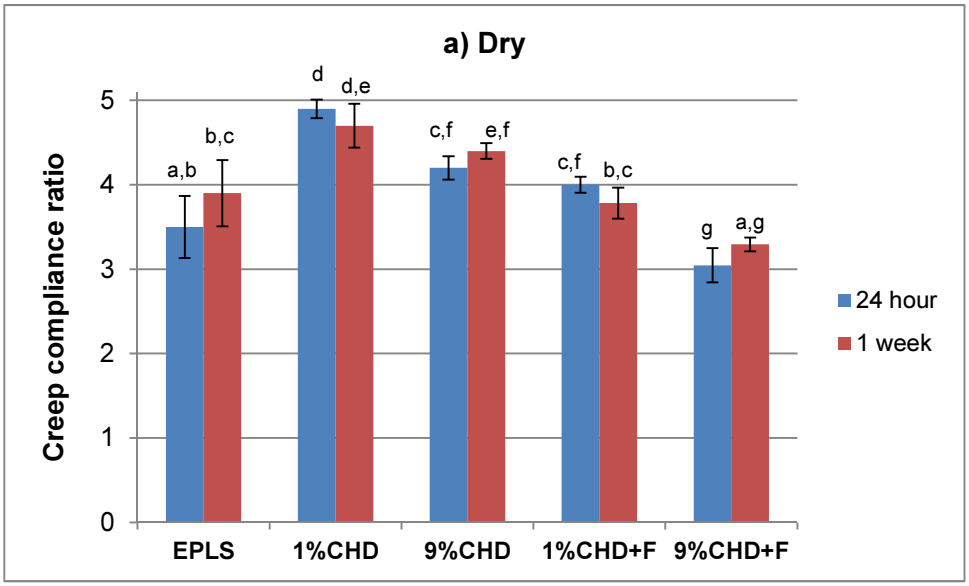

Figure 4.64: Mean (±SD; n=6) CCR of EPLS, EPLS 1% and 9% CHD with and without 0.5% NaF using 30 sec dwell time stored dry at 37°C

(No significant difference between groups with same letters; p≤0.05)

EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml

In DW (Figure 4.65), CCR of EPLS decreased with time from 24 hours to 1 week in all formulations. Addition of 1%CHD increased the CCR but was decreased when CHD was increased to 9% and addition of NaF also decreased the CCR of both 1% and 9%CHD at both time points. There was no statistical difference between EPLS 24 hours and 1% CHD+F 24 hours, similarly 9% CHD 1week and 1% CHD+F 1 week were statistically similar.

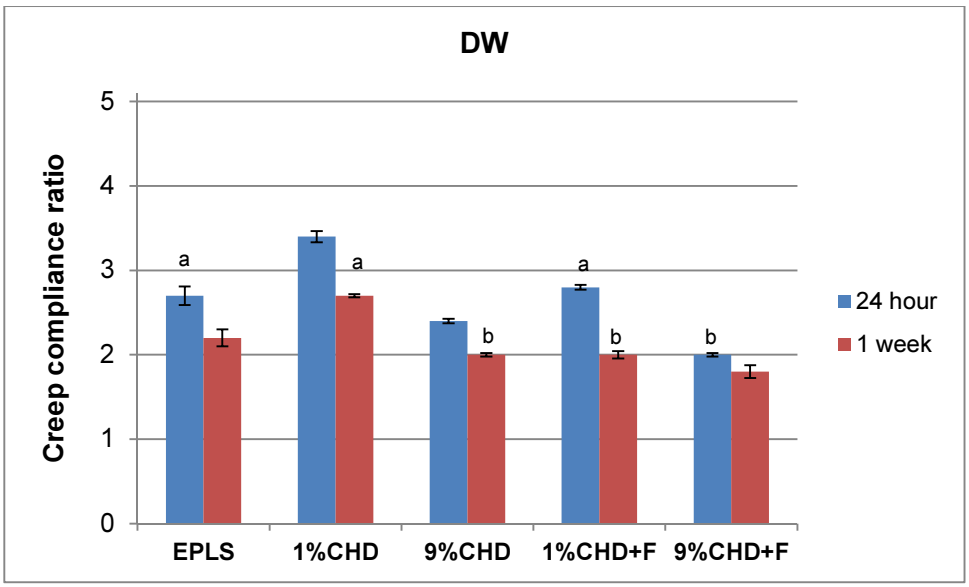

Figure 4.65: Mean (±SD; n=6) CCR of EPLS, EPLS 1% and 9% CHD with and without 0.5% NaF using 30 sec dwell time stored in DW at 37°C

(No significant difference between groups with same letters; p≤0.05)

EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml

In AS (Figure 4.66), CCR decreased with time for EPLS and the two NaF containing formulations whereas it increased for 9%CHD but there was no significant difference for 1%CHD. Addition of 1%CHD had no effect on CCR at 24 hours but increased at 1 week. When CHD was increased to 9% CCR decreased and incorporating NaF also decreased CCR in both 1% and 9%CHD at both time points.

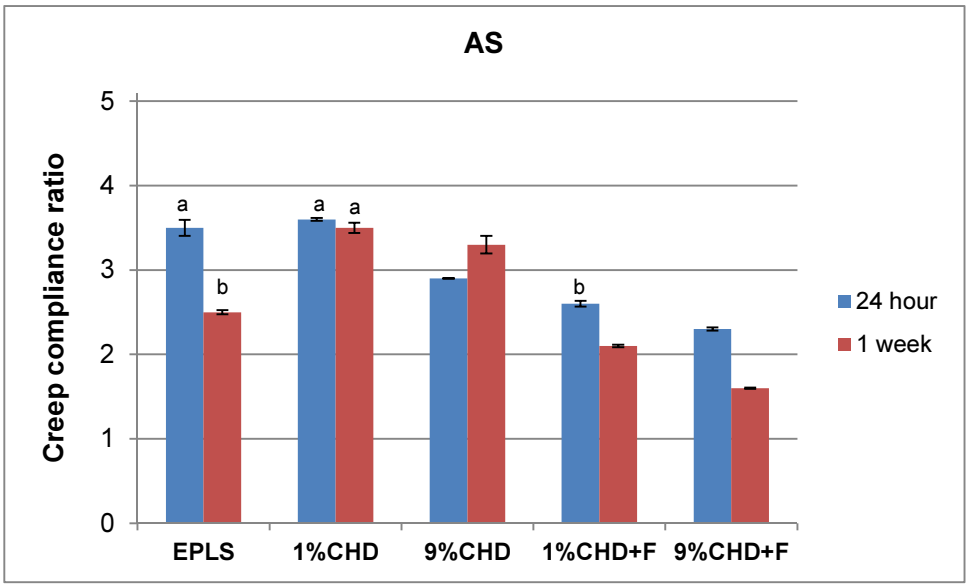

Figure 4.66: Mean (±SD; n=6) CCR of EPLS, EPLS 1% and 9% CHD with and without 0.5% NaF using 30 sec dwell time stored in AS at 37°C

(No significant difference between groups with same letters; p≤0.05)

EPLS: PEMA powder & 95% ATBC+5%ethanol; P/L ratio 1.8g/ml

Figures 4.67 to 4.69 show the mean CCR of EPGS, EPGS 1% and 9% CHD, with and without 0.5% NaF using 30 sec dwell time after 24 hours and 1 week when stored dry, in DW and in AS respectively at 37°C.

Dry (Figure 4.67), there was no significant difference in CCR of EPGS formulations from 24 hours to 1 week except EPGS 1%CHD and 9%CHD where it increased. Addition of 1%CHD increased the mean CCR (was not statistically significant) but decreased when NaF was added after 1 week. CCR was decreased when CHD was increased to 9% but increased when NaF was added after 24 hours but remained statistically constant after 1 week.

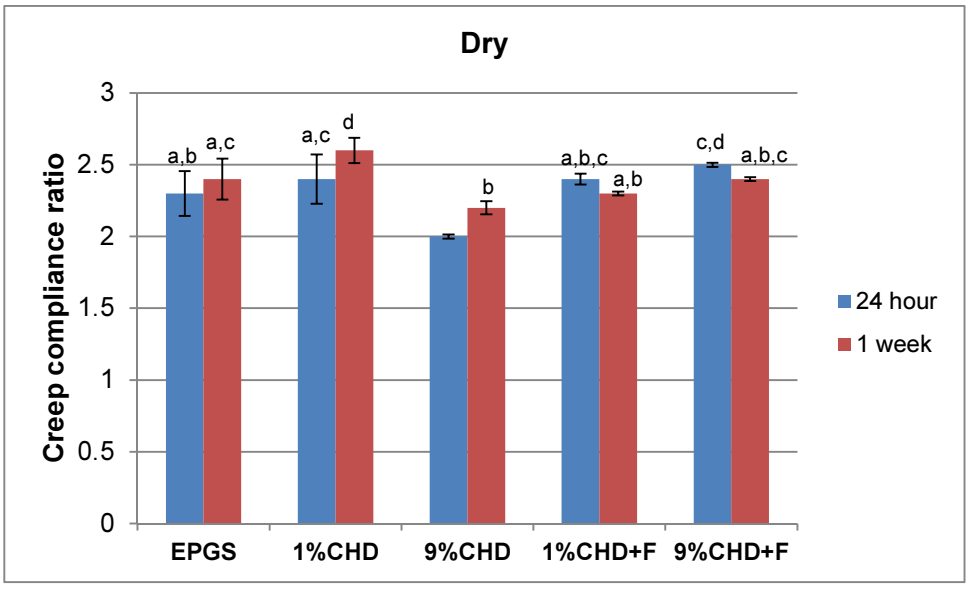

Figure 4.67: Mean (±SD; n=6) CCR of EPGS, EPGS 1% and 9% CHD with and without 0.5% NaF using 30 sec dwell time stored dry at 37°C

(No significant difference between groups with same letters; p≤0.05)
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

In DW (Figure 4.68), CCR of EPGS formulations decreased with time except for 1% CHD and 1% CHD+F where there was no statistical difference. Addition of 1%CHD decreased the CCR of EPGS after 24 hours only but had no effect after 1 week, similarly it was unchanged after 24 hours when CHD was increased to 9% but decreased after 1 week. Addition of NaF increased the CCR both in 1% and 9%CHD, but after 1 week no significant difference was found in 9% CHD.

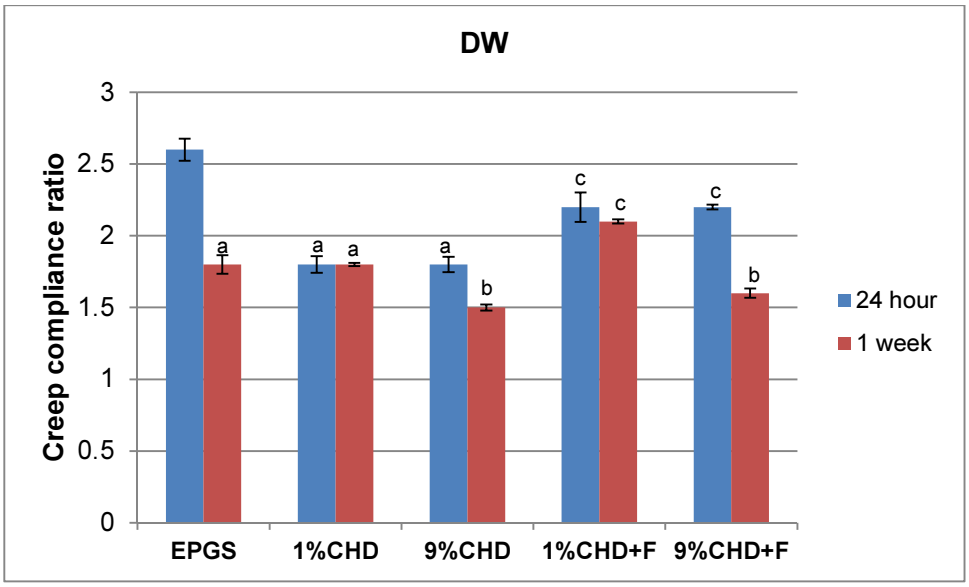

Figure 4.68: Mean (±SD; n=6) CCR of EPGS, EPGS 1% and 9% CHD with and without 0.5% NaF using 30 sec dwell time stored in DW at 37°C

(No significant difference between groups with same letters; p≤0.05)
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

In AS (Figure 4.69), CCR of EPGS decreased with time in all formulations and after 1 week there was no statistical difference between them. After 24 hours addition of 1%CHD increased CCR but its value decreased when CHD was increased to 9% and with the addition of NaF to both. There was also no significant difference in CCR found between 1 %CHD+F and 9% CHD+F after both 24 hours and 1 week.

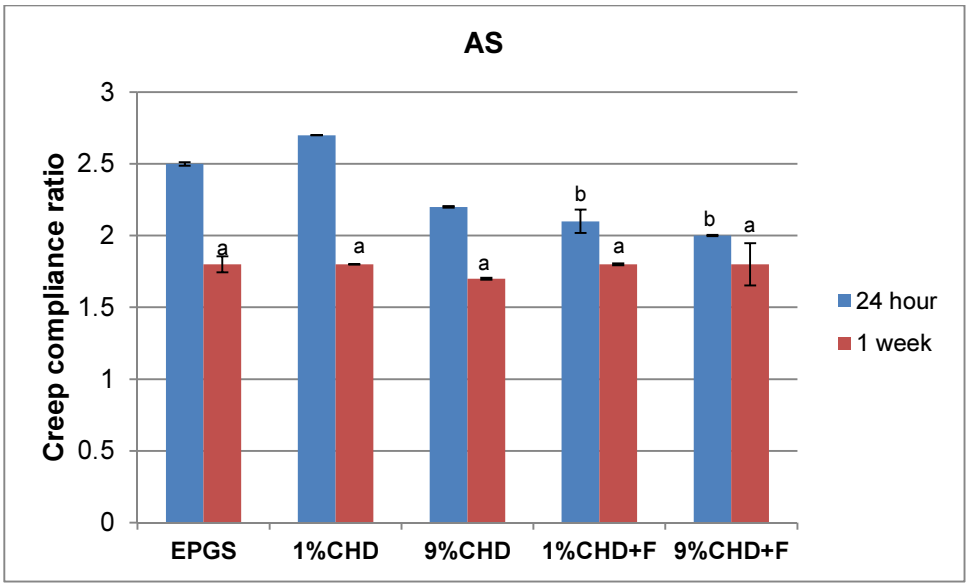

Figure 4.69: Mean (±SD; n=6) CCR of EPGS, EPGS 1% and 9% CHD with and without 0.5% NaF using 30 sec dwell time stored in AS at 37°C

(No significant difference between groups with same letters; p≤0.05)
EPGS: PEMA powder & ATBC; P/L ratio 1.2g/ml

Table 4.14 shows the penetration ratio of VG, EPLS and EPGS containing 1 and 9% CHD with or without 0.5% NaF when stored dry, in DW and in AS after 24 hours and 1 week. At 24 hours the penetration ratio increased (or remained the same) for VG and EPLS when 1% and 9%CHD with or without 0.5% NaF was added, the effect on EPGS was more variable. When stored dry the penetration ratio mainly increased with time, from 24 hours to 1 week, in all formulations except VG where it decreased. When stored in DW and AS the penetration ratio in general decreased with time in all formulations. This reduction was more in AS compared to DW.

Table 4.14: Penetration ratio (R) of VG, EPLS and EPGS; 1 & 9% CHD with or without 0.5% NaF after 24 hours and 1 week

Material	Dry		DW		AS	
	24 hour	1 week	24 hour	1 week	24 hour	1 week
VG 1.8	1.27	1.2	1.22	1.2	1.19	1.2
VG 1%CHD	1.33	1.22	1.30	1.27	1.31	1.25
VG 9%CHD	1.40	1.34	1.40	1.38	1.28	1.33
VG 1%CHD+F	1.41	1.34	1.42	1.27	1.36	1.34
VG 9%CHD+F	1.42	1.42	1.24	1.21	1.29	1.24
EPLS	1.52	1.58	1.41	1.32	1.65	1.42
EPLS 1%CHD	1.74	1.70	1.48	1.48	1.47	1.51
EPLS 9%CHD	1.60	1.62	1.34	1.26	1.46	1.55
EPLS 1%CHD+F	1.55	1.60	1.43	1.25	1.40	1.26
EPLS 9%CHD+F	1.50	1.62	1.30	1.21	1.31	1.16
EPGS	1.36	1.37	1.40	1.26	1.44	1.26
EPGS 1%CHD	1.42	1.49	1.27	1.24	1.51	1.26
EPGS 9%CHD	1.27	1.34	1.25	1.15	1.30	1.22
EPGS 1%CHD+F	1.34	1.36	1.33	1.33	1.25	1.25
EPGS 9%CHD+F	1.40	1.34	1.28	1.20	1.26	1.23

CHAPTER FIVE: DISCUSSION

5 Discussion

Tissue conditioners have been used since the 1960's (Chase, 1961) as chairside materials for application to the patient's denture, cushioning the traumatised tissues, thus allowing them to recover. The P/L tissue conditioner systems contain ethanol as an essential component since it controls the gelation process. However, due to the problems associated with ethanol, as described in the Literature Survey, for example, leaching of ethanol causing irritation to the inflamed mucosa of patients and compromising the materials properties, it would be highly advantageous to develop a tissue conditioner with less or no ethanol. P/L systems also suffer from porosity which can lead to microbial ingress and subsequent fouling. Development of a pre-gelled tissue conditioner excluding ethanol would offer a solution to both problems. Hence this was one of the aims of this project.

There are pre-gelled materials currently available on the market but the majority are for home use, e.g. Snug and Dinabase 7, where they are more popular than the P/L versions. However the majority of them contain large amounts of solvents (Murata *et al.* (2010) and, in the case of Dinabase 7, also require heating prior to application. The development of a pre-gelled tissue conditioner for clinical use, where they can be monitored, would be of obvious advantage.

When developing a new tissue conditioner formulation it is important to have knowledge of its basic properties that make it appropriate for its use. These properties include gelation time, water uptake behaviour, hardness and creep compliance (flow properties). Gelation time is important as these materials are used at the chairside and therefore a short gelation time is desirable. However with a pre-

gelled system this is not important. Due to these materials being used in an aqueous environment it is essential to study their water uptake behaviour and their interaction with the environment in which they are used. Similarly hardness and creep define the materials usage in a particular clinical situation.

5.1 Development of Pre-gelled Systems

The pre-gelled tissue conditioner system developed here did not contain ethanol; the final formulation selected for further investigation was PEMA+ATBC with P/L 1.2 g/ml, referred to as experimental pre-gelled system (EPGS). It was selected based on its low Shore A hardness, high CCR (flow) values and the solubility parameter (δ) of the ATBC being similar to PEMA (the plasticiser is more compatible with the polymer powder). However, it should be noted that preparation of this final system took a considerable amount of time since several problems arose during its development. These are discussed below.

Citrate based plasticisers, namely acetyltri-n-butyl citrate (ATBC) and butyryl tri-n-hexyl citrate (BTHC) were selected for developing the experimental pre-gelled materials, since they are more biocompatible (Nishijima *et al.*, 2002; Johnson, 2002) than some other commonly used plasticisers. Furthermore, Dhiman (2004) recommended the use of these two plasticisers in tissue conditioners. It was anticipated that these plasticisers would naturally gel with the PEMA powder. The weight average molecular weight (M_w) and molar volume of both citrate plasticisers (BTHC and ATBC: 514 g/mol and 504 cm^3; 402 g/mol and 372.6 cm^3 respectively) were compared with butyl phthalyl butyl glycollate (BPBG), a commonly used plasticiser in commercial tissue conditioners (Takamata *et al.*, 2007); (336 g/ml and

300.7 cm^3). The higher molecular weight and molar volume of BTHC and ATBC would mean that these plasticisers may experience more difficulty in penetrating the polymer chains so in turn they will slow the gelation process; however, leaching into the oral environment and subsequent hardening may be reduced.

ATBC and BTHC were mixed with PEMA powder in different powder/liquid (P/L) ratios respectively; all ATBC formulations formed a coherent gel after 16 hours whereas BTHC failed to do so. Mixtures of ATBC and BTHC in three percentages (70:30, 50:50 and 30:70 v/v) were also tried. However, 30:70 ATBC and BTHC failed to form coherent gels after 16 hours at 37°C. To accelerate gel formation the temperature of BTHC based gels was raised to 75°C, which was above the T_g of PEMA. At high temperatures the intermolecular forces in the polymer chains reduce and they become more mobile. It was anticipated that this may facilitate plasticiser ingress and gel formation, but again no coherent gels were formed within 16 hours. This may be attributed to BTHC's higher molecular weight and molar volume compared to ATBC and BPBG, as discussed subsequently. The addition of ethanol, or more time, may be required to facilitate the gelation process, both of which were thought as being not feasible for commercial production. However, further tests (hardness and creep) were carried out before selecting the appropriate final experimental formulation

Dhiman (2004) investigated a range of citrate plasticisers in tissue conditioners formulations and reported that formulations containing BTHC had longer gelation times compared to the other citrate plasticisers used. Other authors have also reported that plasticisers with higher molecular weights /molar volume slowed the

gelation process (Parker and Braden, 1990; Li, 2007; Jones *et al.*, 1988; Murata *et al.*, 2005).

Shore A hardness was used to screen the pre-gelled formulations in the pilot study. The Shore A hardness of the pre-gel systems, containing ATBC only, showed that increasing the P/L ratio increased the Shore A hardness, which is typical behaviour of tissue conditioners as seen in previous studies (Dhiman, 2004; Ali, 2010). Creep compliance ratio (CCR) of these formulations decreased with increasing P/L ratio. This enables tissue conditioners with a range of properties to be produced for use in different clinical situations such as temporary soft lining material and tissue conditioner. Mixtures of ATBC and BTHC for the pre-gelled systems had a higher Shore A hardness and lower CCR compared to the counterpart containing ATBC only. There was no change in CCR in 30:70 and 50:50 ATBC and BPBG formulation. Based on these preliminary pilot studies, EPGS emerged as a suitable pre-gelled material and was investigated further with respect to other physico-mechanical properties.

For EPGS to be a viable commercial material it should have stable physical properties on storage (shelf life) and this was assessed based on the stability of the Shore A hardness results. When stored at 23°C (room temperature) there was a constant increase in the Shore A hardness up to 18 months. However when stored at 7°C (refrigerated) the materials stabilized after 2 days, and the hardness remained constant with no significant difference ($p \leq 0.05$) over 18 months. A possible explanation for this could be that at 23°C there is continuing entanglement of the polymer chains within the gel with time (Parker and Braden, 1990; Murata *et*

al., 2005), thus resulting in an increase in Shore A hardness. When stored at 7°C the mobility of the chains may be greatly decreased due to the lower temperature.

5.2 Particle Size Analysis

Particle size of the PEMA powder in tissue conditioners is important as it influences gelation time and thus handling characteristics. As can be seen from Table 4.2 the PEMA used in VG had a smaller average particle size D[v,0.5] than the PEMA powder used for EPLS (despite being ball milled) and for EPGS (un-milled). However ball milling has been shown not only to reduce particle size, as it did in this study, but also to change particle shape.

Dhiman (2004) used scanning electron microscopy to investigate the effect of ball milling on PEMA powder. He showed that after 16 hours ball milling the resulting powder had smaller, but also, more irregular shaped particles compared to un-milled, which were spherical. He went on also to show that ball milling PEMA powder decreased gelation time of the resulting tissue conditioners, this effect was also demonstrated by other authors (Parker and Braden, 2001; Dhiman, 2004; Li, 2007). Parker and Braden (2001) also attributed the decrease in gelation time to increased surface area of the ball milled PEMA in addition to the decrease in particle size. They proposed that ball milling the polymer powder of a tissue conditioner can be used to enable ethanol content to be reduced while maintaining an adequate gelation time. Li (2007) demonstrated this where mixing a liquid containing ATBC with un-milled PEMA powder gave a gelation time of ~85 min but using PEMA powder ball milled for 16 hours decreased the gelation time to ~18 min. Thus EPLS was formulated using a ball milled PEMA powder.

5.3 Gelation Time

Gelation time for the commercial materials (VG and CC) was measured using the recommended P/L ratio and a higher P/L ratio of 1.8g/ml. The latter was due to the manufacturers suggesting that increasing the P/L ratio of the mix will give a stiffer mix and will speed up the gelation process. The results from this study agreed with this suggestion where increasing the P/L ratio decreased the gelation times.

The gelation process begins when the powder and liquid are mixed together and the polymer beads start to swell in the presence of ethanol to allow the penetration of the plasticiser molecules resulting in gel formation. Thus the presence of ethanol acts as an accelerator to the gelation process (Parker and Braden, 1990). In a study by Parker and Braden (1996) ethanol content was found to have the most significant effect on gelation compared to molar volume of plasticiser, particle size and degree of ball milling the powder. Other factors that can affect gelation time are, for example, amount of ethanol, ball milling of polymer powder, P/L ratio and temperature.

Gelation times (Figure 4.5) for VG and CC decreased with an increase in P/L ratio. The differences in gelation time between VG 1.8, CC 1.8 and EPLS (all with the same P/L ratio) were due to ethanol content (EPLS contained less ethanol) and different plasticiser in CC. The liquid of the latter contained a mixture of benzyl benzoate (plasticiser; M_w = 212.2 g/mol), ester stearic acid (M_w = 284.4 g/mol) and 6.2% ethanol in the liquid (Takamata *et al.*, 2007).

When comparing the gelation time results of VG and CC with other studies it was found that gelation time of CC ranged from ~46.1 min to ~16 min (Graham *et al.*, 1991a; Murata *et al.*, 1997; Dhiman, 2004) and VG from ~6 min to ~16 min (Graham *et al.*, 1991a; Murata *et al.*, 1997; Parker and Braden, 2001; Murata *et al.*, 2001a; Dhiman, 2004; Parker and Braden, 1996). The mean gelation times of CC and VG found in this study were 24.5 min and 11.8min respectively; these are within the range of the results obtained in other studies. The variation of results in different studies can be attributed to the variation in measuring techniques (weight versus volume to dispense powder and liquid), P/L ratio and temperature. Also commercial materials are continuously been changed to improve their properties and it should be noted that VG in previously reported studies contained BPBG as the plasticiser. No other studies have been published which focused on VG formulations containing ATBC.

The recommended composition by Li (2007; i.e. 16 hours ball milled PEMA powder with 95%ATBC and 5% ethanol) was used for EPLS. However the gelation time reported by Li was 15.5 min, whereas in this study the gelation time was found to be 22.5. This is more likely to be due to the variation in molecular weight distribution of the polymer powder used in these studies.

The gelation time of VG was not affected by the addition of CHD at either level, or when NaF was added. The gelation time of EPLS was increased significantly with the addition of 1%CHD, but further addition of CHD to 9%, or incorporation of NaF at both levels, had no further effect. Ethanol content is known to be a major factor in determining the gelation time (Parker and Braden, 1996; Murata *et al.*, 1993). Therefore it can be assumed for VG, which has a higher ethanol content, gelation

will be less affected by other factors. EPLS, with a lower ethanol content, may be influenced more by other factors including addition of CHD and NaF.

5.4 Water Uptake Study

Tissue conditioners are used in the oral environment which can vary greatly in terms pH, temperature and with food and liquid intake. When developing new formulations, since it is difficult to mimic the oral environment in the laboratory, it is critical to understand the materials interaction with, for example, distilled water, and how this affect their physical properties. DW acts as a useful guide to study the diffusion processes without the complications of osmotic effects of the constituents of the immersion solutions as would be found in AS.

In an aqueous environment water diffuses into the tissue conditioner and at the same time some components (plasticiser and ethanol, if present) leach out; the material will harden with time (Braden, 1970a; Jones *et al.*, 1988; Parker and Braden, 1990). The leaching of these constituents is assumed to be the opposite of the gelation process where they penetrate the polymer chains during gel formation (Jones *et al.*, 1988). The change in weight of the tissue conditioner will indicate which process is predominant (Liao *et al.*, 2012).

The water uptake of VG, EPLS and EPGS formulations was measured in DW, at 37°C, over 12 weeks (3 months), to cover the time they would function as temporary soft lining materials, and for 4 weeks (1 month) for those containing CHD and

with/without NaF, to behave as short term tissue conditioners (Graham *et al.*, 1991b).

There was an early rapid weight loss seen only in VG in the first 24 hours, followed by a continued weight loss and a final weight change of ~-5.77% after 3 months. Its behaviour was not in line with previous studies where Addy and Handley (1981) reported ~2% uptake (at ~87 days which is a similar time period to the present study), Murata *et al.* (2001b) reported ~3.5% uptake after 21 days, Sample (2001) reported ~3.4% in 140 days and Dhiman (2004) reported ~2.9% after 160 days immersion DW. The difference between previous studies and this present study is probably due to the change in composition of the VG liquid. Previously, the latter contained BPBG as a plasticiser but this has now been substituted by a citrate-based plasticiser (Dentsply, 2014). Due to the latter, additional changes to the formulation may have been necessitated (e.g. ethanol content to maintain handling characteristics). Hence, change of plasticiser appears to have affected the water uptake characteristics. This is further supported by the percentage weight change results obtained in this project for VG Old (containing BPBG) and VG (containing a citrate plasticiser). The former presented with an uptake of ~1.2% for the 1.3 P/L ratio and ~ 9% for the 1.8P/L ratio compared with ~-5.7% for the VG 1.5 P/L ratio and ~-0.9% for the 1.8 P/L ratio.

As mentioned earlier, ATBC has a higher molecular weight and molar volume compared to BPBG. Researchers have suggested that lower molecular weight plasticisers can leach out more easily compared to higher molecular weight plasticisers (Murata *et al.*, 2001b; Sample, 2001; Dhiman, 2004; Hong *et al.*, 2012). This was not the case for VG in this study as explained above. It could be argued

that ATBC is aliphatic and a linear molecule whereas BPBG is aromatic, containing a benzene ring. Therefore, it may have been easier for ATBC to penetrate the polymer chains, and its linear structure may have facilitated its leaching.

The weight loss of VG was lower when the P/L ratio was increased from 1.5 g/ml to 1.8 g/ml because increasing the P/L ratio reduced the amount of liquid present, thus the amount of ethanol and plasticiser present in the material. This therefore led to a lower weight loss at this ratio. On comparing the two experimental formulations EPLS (P/L) and EPGS (pre-gelled), the former initially gained weight and then lost weight in the first 24 hours, whereas the latter gained weight during this period. The differences in the initial uptake profiles can be attributed to the loss of ethanol and some plasticiser from EPLS. Hence, the effect of ethanol content has been confirmed by the varying weight change profiles in the first 24 hours for VG (containing the highest amount of ethanol), EPLS (containing 5% ethanol) and EPGS (containing no ethanol) (see appendix A1). Similar findings of ethanol being lost in the first 24 hours, have been reported by (Jones *et al.*, 1988). In EPLS the ethanol loss facilitated plasticiser loss (Parker and Braden, 1990; Murata *et al.*, 1994). Some researchers have reported that materials containing more ethanol lost more weight due to it being more soluble in water and having a small molecular size compared to the plasticiser; therefore it will leach easily (Braden and Causton, 1971; Murata *et al.*, 1996; Dhiman, 2004).

When comparing the final weight change of VG with EPLS and EPGS, the former presented with negative weight loss whereas both EPLS and EPGS had a net weight gain. This can be attributed to the differences in compositions of the formulations of the three systems (e.g. ethanol content and P/L ratio) and the

differences in particle size of the polymer powders used. Overall, the weight changes seen are a combination of water being absorbed by the samples and simultaneously, ethanol and plasticiser leaching. The higher weight increase of EPGS could be due to a lower P/L ratio (1.2g/ml) and thus higher plasticiser content compared to EPLS. In addition there was no ethanol in EPGS so there was no early weight loss. Hence, ethanol and plasticiser leaching form VG is the predominant process whereas in EPLS and EPGS water uptake seems to be the predominant process.

Addition of 1% CHD enhanced the weight changes in all formulations of tissue conditioners. VG showed an increased weight loss whereas EPLS and EPGS showed increased weight gains. On increasing the CHD content from 1% to 9% increased i) the weight loss in the case of VG, ii) the weight gain in the case of EPGS, and iii) no significant change ($p \leq 0.05$) for EPLS. The further addition of 0.5% NaF to both 1% and 9% CHD increased the weight loss in VG and weight gains in EPLS and EPGS. This is because CHD and NaF acted as water soluble impurities and hydrophilic sites and for droplet formation. The osmotic pressure of the solution in these internal droplets increased until they balanced the restraining force of the material, thus leading to higher water uptakes. Although the presence of CHD in the systems increased the water uptake, the further addition of NaF had a much greater effect due to its high solubility and low M_w (Sample, 2001). This means NaF solutions formed higher osmotic pressures than CHD (Patel *et al.*, 1998) even though the incorporated weight (%) of the former was low.

The results found here are in line with other studies where addition of CHD increased the water uptake of the materials, and this was further enhanced with the

addition of NaF. Parker *et al.* (1997a) reported the effect of CHD incorporation on gels containing PEMA with 10% ethanol and BPBG immersed in DW for 4 weeks at 37°C. Addition of 0.9% CHD gave a weight change of 2.5% and, when 9% CHD was added, this increased to 9.6% weight change. Sample (2001) reported that the % weight change for experimental tissue conditioners, with a similar composition to Parker *et al.* (1997), had 4.1% and 14.1% weight change for 0.9% and 9% CHD containing materials respectively. When 0.5% NaF was added to 0.9% CHD the weight change (%) increased to 36.1%. Similar trends were reported by Hassan (2007), where the weight change (%) after 2 weeks immersion in DW at 37°C, of PEMA mixed with 95% ATBC and 5% ethanol increased from 2.1% to 7.5% when 1%CHD was added and, the uptake was further enhanced to 32.6%, when 0.5% NaF was added. In this study EPLS had the same composition as Hassan's materials and its % weight changes after 2 weeks were at a similar level of 6.5% for 1%CHD and 30.3% for 1%CHD+F.

Generally all water uptakes plots against $t^{½}$ appeared linear, with the exception EPGS 9%CHD+F and EPGS1%CHD+F, the latter material being concave to the $t^{½}$ axis. To determine whether the weight changes (%) were following Case II diffusion in the latter material, the water absorption data were plotted against time. However, the plots proved not to be linear. Therefore it can be concluded that in the presence of sodium fluoride the water uptake process for EPGS became complex and was anomalous (i.e. non-Fickian).

Due to the complex nature of the uptake profiles, in order to get a better understanding of the processes involved, the water absorption data for all systems was analysed further for evidence of Fickian diffusion as described subsequently. If

the data is plotted as log uptake against log time (Eq 6.1) and a linear relationship is presented, the gradient of the linear region will determine if the process is Fickian. Hence, if the gradient is ~0.5, then the uptake behaviour is classed as Fickian (Sample, 2001)

$$uptake = kt^{1/2} \qquad \textbf{Eq 6.1}$$

then

$$log\ uptake = \frac{1}{2} logt + logk \qquad \textbf{Eq 6.2}$$

Figure 5.1 shows a typical example of a log weight change (%) against log time (in seconds) plot; the rest of the plots are displayed in the Appendix. EPLS, EPLS 1%CHD, EPGS and EPGS1%CHD+F gave gradients less than 0.4 showing that diffusion behaviour was not Fickian in nature. All other formulations yielded a gradient between 0.4 and 0.6, and hence indicate that uptake behaviour was Fickian. It could be argued that because tissue conditioners are soft materials a typical swelling behaviour is seen during water uptake compared to crack formation in rigid polymers (Parker *et al.*, 1997a; Patel and Braden, 1991; Parker and Braden, 1989). Also, the crosslinks between the polymer chains are time dependent because there is no chemical reaction in the formation of the gel, compared to polymerised polymers. All these factors affect the diffusion behaviour.

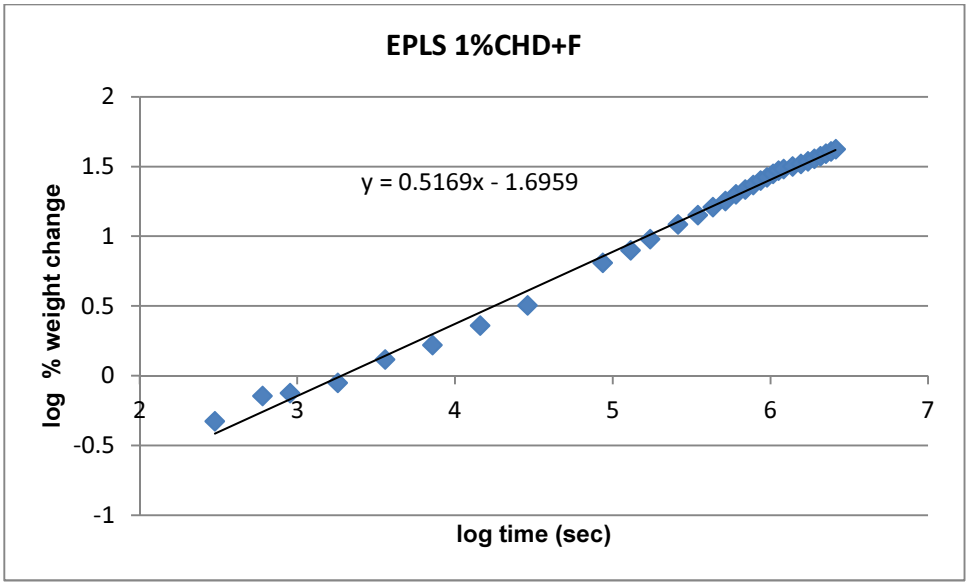

Figure 5.1: log of % weight change of EPLS 1%CHD+F against log time (sec)

After the water uptake studies the materials were desorbed at 37°C for 1 week. All formulations showed similar weight loss profiles presented as a rapid weight loss in the first 24 hours, followed by equilibrium within a period of 1 week, as reported in other studies (Hassan, 2007; Dhiman, 2004; Sample, 2001). All $t^{½}$ plots showed an initial linear region thus indicating that water desorption was Fickian in nature Also for diffusion to be Fickian, plots of M_t/M_∞ (weight of sample at time, t, divided by equilibrium weight of sample) against the square root of time should yield a straight line (Patel and Braden, 1991). The data was re-plotted in this manner to obtain the slope, which was then used to calculate the diffusion coefficients for the desorption process (D_{des}). Generally D_{des} values increased when CHD amount was increased in the formulation from 1% to 9% (except for EPLS) and when NaF was added (except VG 9%CHD). Interestingly, it should be noted that D_{des} was in the region of 10^{-10}sec^{-1} for EPLS, EPGS and VG Old and slower for VG (10^{-11} m^{2}sec^{-1}). Again this is a reflection of the amount of ethanol in the latter.

This is further confirmed by the solubility results, which show that VG formulations had very high solubility values compared to VG Old, EPLS and EPGS. Similarly, solubility and real uptake (%) were increased when CHD was added and when the amount was increased from 1% to 9%; the effect was further enhanced when NaF was added. It is again assumed that is due to the higher ethanol content in VG and experimental formulations. All real uptake values were high for all formulations containing additives (CHD and NaF). This also suggests that as the water was drawn into the materials, the droplets around impurities/additives continued to grow, due to creep, until the restraining forces on the droplet by polymer matrix, equalled the osmotic pressure difference between the external solution and the droplet (Sample, 2001). It should be noted that VG old 1.3P/L presented with a negative solubility (-4.3 ± 0.3), indicating that the material gained weight after desorption. This could mean that the surfaces of the samples were weakly bonded with impurities. Although this finding has not been reported before in the literature for tissues conditioners; similar findings have been reported for water uptake of dental composites from AS (Mustaza *et al.*, 2014).

5.5 Chlorhexidine and Fluoride Release

CHD was added to the formulations as 1% and 9% by weight of the total mix, where 1% CHD showed a higher %CHD (but lower weight) release compared to the formulations containing 9% CHD; this release was further enhanced when 0.5% NaF was incorporated to both levels of CHD. Several factors affect the release of CHD from these materials. CHD is soluble in ethanol (1 part CHD is soluble in 15 parts of 96% ethanol; MSDS-CHD, 2011; (Sigma, 2011)). Therefore the formulations containing more ethanol should release more CHD. This proved to be the case where VG formulations (containing the highest amount of ethanol) presented with

higher CHD release followed by EPLS (containing 5% ethanol) and then EPGS (containing no ethanol). Another possible mechanism by which ethanol may facilitate CHD release is by the formation of paths or other local structural changes that would facilitate leaching (Sample, 2001). This theory seems unlikely, given the viscoelastic nature of these materials; even if paths were developed due to leaching of ethanol, they will not remain as permanent plastic deformations in the material. It is also logical to assume that a higher level of CHD will be released first from the surface and, subsequently to a lesser extent decreasing with time, from within the gel. This was evident from the amount of CHD released where more was released in the 1st week compared to the subsequent three weeks, both in terms of actual weight and percentage.

Another factor that may affect CHD release is the pH of the immersion solution. It has been shown by different studies that more CHD is released in acidic solutions compared to neutral solutions, because the solubility of CHD at low pH is higher (10.4 g/L at pH 4) than at higher pH (3.6g/L at pH 6) (Shen *et al.*, 2010; Anusavice *et al.*, 2006). The normal pH of the oral cavity ranges between 6.2 to 7.5 (Aframian *et al.*, 2006), which can vary according to food or drink intake, so more CHD would be released if the oral cavity was slightly acidic compared to a neutral pH. When fresh DW comes in contact with air, carbon dioxide dissolves in it forming carbonic acid that gives the DW a pH between 5 and 6. A study conducted by Uddin (2014) reported the pH of DW as 6.1±0.3; this same source of DW was used in this study, so this may result in higher release of CHD from the materials.

Parker *et al.* (1997a) reported CHD release as 1.14mg and 10mg from 0.9% and 9% CHD by weight respectively from a tissue conditioner containing PEMA with 10%

ethanol and BPBG after 4 weeks in solution whereas EPLS 1%CHD and 9%CHD showed a release of 2.8mg and 9.27mg in 4 weeks, comparing both results a similar release is seen after 4 weeks although ATBC was used in the current studies with 5% ethanol. Sample (2001) reported 0.82mg and 9.86 mg of CHD release respectively with the same composition used by Parker *et al.* (1997a) but over 120 days. Sample (2001) also reported that tissue conditioners containing BMA/EMA with 2% ethanol and ATBC released 0.51mg and 6.98mg CHD when 0.9wt% and 9wt% CHD were incorporated. The compositions containing more ethanol released more CHD, which is in agreement with the trends seen in this study. Furthermore, when NaF was added to 0.9% containing CHD, release was increased from 0.51mg to 2.94mg. Again the same effect of enhanced release with the addition of NaF was seen in this study. This might be attributed to the fact that CHD is positively charged and F is a negatively charged ion, so during water absorption chlorhexidine difluoride is formed (Shen *et al.*, 2010). Also NaF increases the water uptake and swelling of the materials thus facilitating a higher amount of CHD release as indicated by the higher solubility values shown in Table 4.4.

It was noted that initial %CHD release was rapid followed by a slower rate of release. To investigate further the gradient of the %CHD release against $t^{1/2}$ after 24 hours to 1 week was calculated with the intercept with the y-axis taken as the initial burst release. Comparing gradients VG was higher than EPLS both much higher that EPGS, related to ethanol content as discussed earlier. Averaging the gradients of VG, EPLS and EPGS formulations with the same additive to try and identify relative magnitudes of effects showed the same trends. For all materials 1%CHD showed faster release than 9%CHD however the weight of CHD released was higher. This means a higher concentration of CHD is present in immersion solution leading to lower concentration gradient resulting in less release. Although there is

more CHD in the 9% CHD material not all will be in the solution droplets formed at the CHD sites, which is limited by water solubility of CHD (MSDS-CHD, 2011; Sigma 2011). Change in the immersion solutions at 1 week and 2 week does not seem to have an effect on the CHD release.

When looking at the gradients and intercepts only trend seen is that higher release rates (gradients) show negative intercepts indicating no burst release. For early or burst release it may be better to look at Table 4.5 which shows the weight and %CHD released in the first 24 hours. In this case VG releases more than EPLS and much more than EPGS. This can be attributed to ethanol content which is said to leach out rapidly with some studies reporting all being lost with in the first 24 hours as reported by by Jones *et al.* (1988). Considering the percentage CHD release again 1% CHD materials are higher than the 9% materials but by weight it is the other way round. However the weight release is not proportionally higher than the 1% CHD materials which may be related to the solubility in ethanol as previously noted in this section.

The release of CHD from tissue conditioners is complicated owing to the simultaneous loss of ethanol, plasticiser and also high water uptake. This is particularly evident in VG formulations with overall loss in weight negative weight change and resulting in high solubility (Table 4.4).

To analyse the release mechanisms from these materials log of % CHD release was plotted against log of time (as explained earlier). The plots showed that CHD was released in two phases both with different mechanisms. The initial release phase was rapid followed by a slower release in the second phase, as shown in Figure 5.2, as a typical example (the remaining log/log release plots can be found in the Appendix). In the initial linear phase only VG 1%CHD, VG9%CHD, EPLS 1% CHD, EPLS 9%CHD+F had gradients between 0.4 and 0.6, thus indicating a Fickian release process. CHD released from the initial phase of all the other formulations, and the second phase of all formulations, was by non Fickian kinetics since the gradients were less than 0.4.

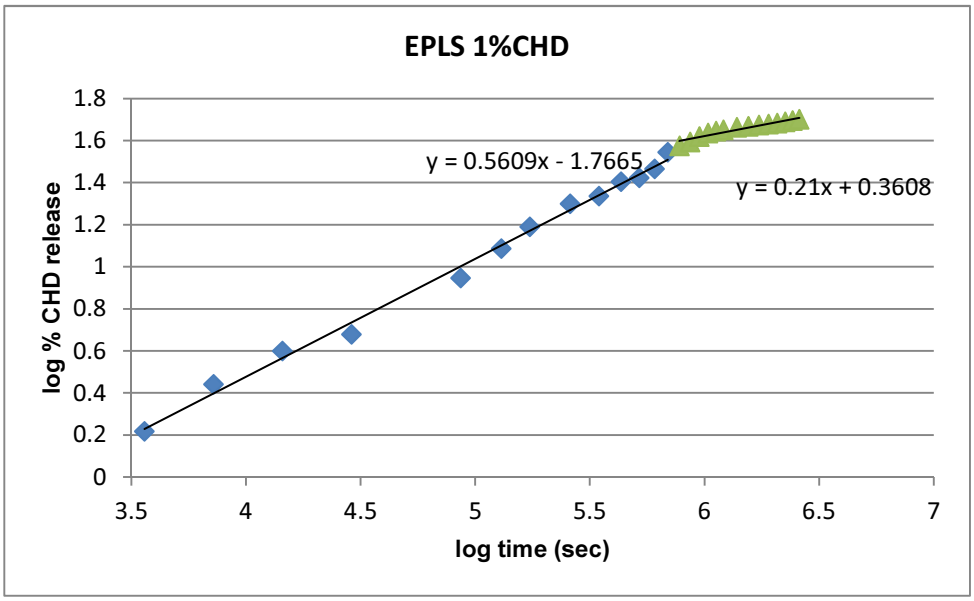

Figure 5.2: Log % CHD release of EPLS 1%CHD against log of time (sec)

NaF was added to the formulations to enhance release of CHD and not to provide a therapeutic effect, so only a small amount (0.5%) was used. The same percentage has previously been used in different studies which have been shown to have a similar effects (Patel *et al.*, 1998; Sample, 2001; Hassan, 2007). From the results summarized in Table 4.7 the amounts of F released were similar to the amounts

found by Sample (2001). After 4 weeks immersion in DW, Sample (2001) found that formulations based on BMA/EMA copolymer powder with 2% ethanol and ATBC showed a release of 2.21 ppm. Here VG 1%CHD+F showed a release of 3.86 ppm whereas EPLS and EPGS 1%CHD+F showed releases of 2.65 and 2.36 ppm respectively, which are similar to the findings of Sample (2001). The difference with VG is most probably due to the differences in polymer powder and the ethanol content.

When the gradient of the %F release against $t^{1/2}$ after 24 hours up to 1 week was calculated with the intercept with the y-axis taken as the initial burst release for further analysis, the %F release rate of 9%CHD was higher than 1%CHD for VG and EPLS but similar for EPGS. Again negative intercept for higher release rates indicates absence of burst release but again considering F release after 24 hours shown in Table 4.8 the 9% CHD+F materials all released more F than 1% CHD materials both measured as ppm and percentage. As was previously noted F is probably released as chlorhexidine difluoride and the 9% CHD materials release a higher weight of CHD (Shen *et al.*, 2010).

Generally, in all formulations, increasing the content of CHD increased the level of F released at 1 day, 1 week and 4 weeks. It proved difficult to analyse the process by which fluoride ions were released. Log F release against log time plots for all formulations are displayed in the Appendix, where none of the plots initially released fluoride by a diffusion controlled process. Therefore it can be concluded that fluoride was released by an anomalous process.

Comparing water uptake and %CHD release profiles, it was noted that there appears to be an arrest in both which occurs around 100 - 200 $sec^{1/2}$. In the uptake profiles the weight change is due to a combination of inherent matrix uptake and also weight loss particularly due to ethanol loss (Braden and Causton, 1971; Murata *et al.*, 1996; Dhiman, 2004). Afterwards the change is slope is due the water reaching CHD/F sites and formation of solution droplet (Dhiman, 2004). Then the uptake is driven by the osmotic gradient and droplet growth (Liao *et al.*, 2012; Muniandy and Thomas, 1984). This will also influence the CHD/F release rates (Sample, 2001). Again due to the complex water uptake and loss of plasticiser and ethanol as well as CHD and F the release of later will affect the osmotic gradient. The change of immersion solutions at 1 week and 2 week, however, does not appear to have much of an effect on uptake or release except on formulations containing F where higher release is seen so saturation is more likely to occur.

5.6 Shore A Hardness

Shore A hardness is commonly used for elastomeric materials although ISO 10139-1, ISO 10139-2 (2009) standards for soft lining materials recommend IRHD. The main differences between Shore A testing and IRHD are that Shore A uses variable (spring load) force and is used for sample thickness of 6 mm or more of whereas IRHD uses a constant (dead load) force and is used for sample thicknesses of 4mm or above. Additionally, the dwell time in IRHD is an initial minor load for 5 sec and then a secondary load for 30 sec, a total time of 35 sec (Hertz and Farinella, 1998) whereas for Shore A hardness, the standard dwell time is 1 sec (ASTM D 2240-05; 2005). Thus, IRHD hardness measurements can be effected by creep due to the extended dwell time, whereas this effect will be minimised using a dwell time of 1 sec.

ASTM D2240 (Testing and Materials, 2005) standard for Shore A hardness recommends a minimum thickness of 6mm, however this is not practical in clinical situations. According to Murata et al (2009), tissue conditioners are optimally compliant when used in a thickness of 1.5-2 mm, a more clinically appropriate thickness. The thickness used in this study was 10mm and, although it may not be relevant clinically, a lower thickness is not suitable for materials with very low Shore A hardness values (<20). In studies by Siddiqui *et al.* (2010) and Ali (2010) the effect of increasing the thickness resulted in decreasing hardness. It was found that when thickness was increased from 1 to 6mm there was a decrease in Shore A hardness and then the values remained almost constant when the thickness was increased from 6mm upwards. Ali (2010) showed that the Shore A hardness of a experimental tissue conditioner formulations containing PEMA+BPBG or PEMA+ATBC decreased more than 10 fold when thickness was increased from 1 mm to 6 mm. Additionally Siddiqi *et al* (2010) found that for materials with lower Shore A hardness the decrease showed more dependence on thickness. So to evaluate the true Shore A hardness of tissue conditioners it was considered that a 10mm thickness is more suitable to ensure there is no effect of thickness.

The commercial materials, VG and CC, were tested using two different P/L ratios. One was the manufacturer's recommended P/L ratio and a higher P/L ratio (1.8g/ml), which was suggested by the manufacturer if a stiffer gel was required, as explained in section 5.3. When VG was developed the original P/L ratio used was 1.8g/ml (Jones *et al.*, 1986); this was also used by Dhiman (2004) in his studies. Additionally, a study was conducted by Yahaya (2003) in the Dental Institute of Barts and London School of Medicine and Dentistry, in which 8 clinicians were asked to mix a tissue conditioner to their preferred consistency. The results varied from 1.1g/ml to 1.8g/ml so, a P/L ratio of 1.8g/ml was also used in this study.

Figure 4.39 shows the Shore A hardness values of all the materials measured 1 hour after mixing. Differences in Shore A hardness between the materials as may be due to many factors as detailed in section 2.6.2. EPGS has the highest Shore A hardness despite a low P/L ratio (Figure 4.39). This is because EPGS, at the time of measurement which, as explained earlier in section 4.9.1, is actually 16 hours after mixing will be in the post gelation phase.

When the P/L ratios of VG and CC were increased the Shore A hardness of the materials also increased. This increase in powder content will reduce the overall level of plasticiser/ethanol in the material and reduce polymer chain mobility. Thus the material will resist penetration of indenter leading to higher Shore A hardness. VG 1.8, CC 1.8 and EPLS have the same P/L ratios but different Shore A hardness values. In this case differences may be due to gelation time where VG 1.8 has the shortest gelation time and EPLS the longest. Thus after 1 hour VG 1.8 is most likely to be in the post gelation stage (Murata *et al.*, 2005) and CC 1.8 and EPLS are still in sol-gel stage (Murata *et al.*, 2005) so gelation may not be complete. Additionally, VG and CC both contain ATBC but VG contains more ethanol. A study by Jones *et al* (1991b) reported that a high ethanol content produced stronger gels which would resist penetration by the indenter and give a higher Shore A hardness value. A number of studies have shown the lower Shore A hardness of CC compared to VG. Dhiman (2004) attributed this to the lower M_w plasticisers in CC.

The Young's modulus was calculated from Shore A hardness, 1 hour after mixing, as explained in section 2.6.2 According to Murata *et al.* (2009) the physical properties of soft lining materials should ideally be equal to the oral/basal mucosa and for tissue conditioners Young's modulus should be less. This is so that when

loaded, e.g. during mastication, the tissue conditioner will deform more than the mucosa so acting as a cushion (Braden *et al*., 1995). Inoue *et al.* (1985) reported that Young's modulus of oral mucosa ranges between 0.4 – 4.4 MPa. The results in Figure 4.42, show that the Young's modulus of EPLS and EPGS were 0.22 and 0.24 respectively and lower than that of oral mucosa as proposed by Murata *et al.* (2009).

As well as continuing gelation/maturation, storage in different media will also affect Shore A hardness with time. The different formulations were stored dry, in DW and in AS at 37°C in order to study and compare the effects of different conditions. When stored dry, the materials will only be influenced by temperature. In DW and AS, where Shore A hardness will be influenced additionally by ethanol/plasticiser leaching and water uptake, AS will also simulate the oral environment.

When stored dry at 37°C (Figure 4.39), Shore A hardness for the different P/L formulations increased over the first 24 hours after which there was only a minimal increase up to the end of experiment (1 week). This suggests that up to 24 hours there is continuing gelation (sol-gel stage) or continuing chain entanglement in the post-gel phase as described by Murata *et al.* (2005). From 24 hours to 1 week the formulations will be mature gels and so only minimal changes in Shore A hardness will occur. EPGS showed the lowest changes over time as it was already a mature gel at the start of the experiment, as previously noted.

When immersed in DW and AS at 37°C (Figures 4.40 and 4.41), an increase in Shore A hardness with time was seen in all formulations, including EPGS. Plasticisers and ethanol will leach out over time resulting in hardening of the

materials,(Ali, 2010). The increase in Shore A hardness with time was higher in DW compared to in AS. AS is an aqueous solution and contains a number of different components that may affect the uptake/leaching processes. Several studies have shown that water uptake is less from solutions than water because of the reduction in osmotic gradient, the driving force for uptake (Dhiman, 2004; Liao *et al.*, 2012). A study by Kazanji and Watkinson (1988) showed that in artificial saliva tissue conditioners have a lower weight change, which then proposed that it was due to less plasticiser loss compared to DW. Yahaya (2003) also showed that when tissue conditioners are immersed in AS, they had lower change in Shore A hardness with time, which again was attributed to less plasticiser loss.

After one week storage in all media EPLS had the lowest Shore A hardness compared to VG 1.8 and CC 1.8 (with the same P/L). EPGS showed the least change over time in all media indicating the stability of the material. Both reflect the differences in ethanol content between the materials where VG has the highest and EPGS the lowest.

In the literature, either Shore A hardness or compliance (which is inverse of hardness) was used to measure the softness of these materials. Murata *et al.* (1996) studied the changes in compliance in different tissue conditioners namely CC, CS, GC and VG, in DW over a period of 28 days at 37°C. They reported that biggest change in compliance was seen during first few days, which agrees with the findings of this study where for VG and EPLS the major changes in Shore A hardness were seen during the first 24 hours.

Yahaya (2003) reported the effect of storage in DW and AS on Shore A hardness of VG and an experimental tissue conditioner containing 50/50 BMA/EMA copolymer with 2% ethanol and ATBC. The Shore A hardness of VG increased more in DW than in AS, however the experimental tissue conditioner showed an increase in Shore A hardness in DW but decrease in AS. This is contrary to the results found in the present study where Shore A hardness increased in both DW and to a lesser extent in AS for both VG and EPLS. This may be attributed to the difference in composition where Yahaya's experimental material had lower ethanol content at 2%, compared to VG and EPLS leading to lower plasticiser loss (Jones *et al*, 1988), as shown by the higher solubility in DW compared to AS, so reducing the hardening effect Additionally the study was carried out over 5 weeks where the resulting higher water uptake may have a plasticising effect which can partially compensate for plasticiser loss (Murata *et al.*, 2009).

Ali (2010) investigated the effect of immersion on two experimental tissue conditioners containing PEMA powder with 5% ethanol and BPBG or ATBC, the latter being similar in composition to EPLS. The results showed that Shore A hardness increased with time in DW and AS, and increase was higher in DW than in AS. There were also rapid changes in hardness during the first week followed by a gradual increase, supporting the findings from this study

One of the aims of the study was to investigate the effect of incorporation of CHD with and without NaF for VG, EPLS and EPGS on Shore A hardness. In general when CHD and NaF were added to VG, EPLS and EPGS, Shore A hardness was increased. When CHD and NaF were added they would be dispersed within the gel matrix where, as they are particulate, they may act as fillers and increase the

resistance to penetration of the indenter into the material and thus increasing the Shore A hardness (Schneid, 1992).

Shore A hardness measured at 1 hour after mixing generally increased for all formulations when CHD with and without NaF were added with only slight variations. Changes in Shore A hardness over time for the materials containing additives followed similar trends as seen in the original materials but the final values were higher (Figures 4.43 – 4.51). These higher values result from higher solubility values (Table 4.4) for these materials indicating greater plasticiser loss. As for the materials without additives, EPLS formulations had the lowest Shore A hardness after 1 week and EPGS formulations showed the least change. Additionally, EPLS was the only formulation (except 9%CHD) and VG 1%CHD had Young's modulus values at 24 hours (Figure 4.53) below the range for the mucosa (0.4 – 4.0 MPa) found by Inoue *et al* (1985).

There are few studies that have been carried out on the effect of addition of antifungal drugs on Shore A hardness. However, the increase in Shore A hardness when CHD was added into the soft lining materials has been reported by some authors. Urban *et al.* (2014) investigated the effect of incorporation of antifungal drugs into Softone, a tissue conditioner and Truesoft, a resilient liner, on Shore A hardness when immersed in DW at 37°C. The Shore A hardness of both materials increased with time and when the amount of antifungal drugs was increased thus showing the same trends found for EPLS in DW in this study. Similarly Bertolini *et al.* (2014) also investigated the effect of incorporation of CHD into Coe-soft and Truesoft when stored in DW, as found in this study, the Shore A hardness increased with time for both materials. They also found that increase in the amount of CHD did

not have an effect on Coe-soft however, it did increase Shore A hardness of Truesoft. The authors proposed that this is due to the difference in composition between the materials e.g. plasticisers. Similarly in this study, increase in 1%CHD to 9% increased the Shore A hardness for EPLS but decreased it for VG where the main difference is ethanol content.

5.7 Creep Compliance Ratio

Viscoelastic materials show a relationship between stress and strain which depends on time. If the stress is held constant, the strain increases with time (viscoelastic creep); and if the strain is held constant, the stress decreases with time (viscoelastic relaxation). In this study the ratio of compliance at time t (dwell time) to the compliance at 1 sec dwell time is used to calculate the creep compliance ratio (CCR). CCR is a measure of flow in the material and is an important property of a tissue conditioner where it can determine the appropriate clinical application of the material. When used as a tissue conditioner the material should have a high flow (high CCR) so that it can adapt to the underlying tissue and it should maintain its flow so as to allow the tissues to heal. When used as a temporary soft liner they should have relatively low flow (low CCR) maintained throughout the time required in the mouth (Murata *et al.*, 1996). In functional impression material a very high flow (very high CCR) is required at the time of placement so it can record the details accurately but the flow should then reduce quickly so as to maintain the record of the anatomy for a maximum of 24 hours in the mouth (Shylesh *et al.*, 2013).

Shore A hardness was measured using dwell times from 1 sec to 30 sec. All the formulations showed that by increasing the dwell time Shore A hardness decreased

indicating the occurrence of creep. Penetration of the indenter using a dwell time of 1 sec will result in a mainly elastic strain whereas for longer dwell times plastic strain will also occur thus resulting in lowering the Shore A hardness.

As can be seen from Table 4.10, at 1 hour after mixing increasing the P/L of both VG and CC (to a lesser extent) decreased CCR reflecting the decrease in ethanol/ plasticiser content. The CCR for VG and EPLS I hour after mixing (Table 4.10) were much lower than CC which is due to the difference in plasticiser. CC contains lower molecular weight plasticisers which will lead to less polymer chain mobility compared to those with higher molecular weights (Jones *et al.*, 1986; Jones *et al.*, 1988). The very high CCR of EPLS may also be due to continuing gelation at 1 hour time thus resulting in higher flow (high CCR). The high CCR found for VG at 1 hour may result from higher ethanol content so facilitating polymer chain mobility (Murata *et al.*, 2001a). However, both VG and EPLS showed a dramatic decrease in CCR at 24 hours in all storage media when they would have reached the post gelation stage. After 24 hours there was minimal change in CCR for all materials in all storage media up to 1 week (Table 4.11). EPGS had the lowest CCR of all formulations as it was measured 16 hours after mixing as previously discussed so was a mature gel. This is further evident by the fact that there was no statistically significant ($p \leq 0.05$) change in CCR throughout the 1 week of the study, when stored dry (Table 4.11).

Considering the ATBC containing materials, the order of decrease in CCR was VG<EPLS<EPGS which equates inversely with ethanol content which is in the order VG>EPLS>EPGS.

According to ISO 10139-2 the penetration ratio (R) of 30 sec and 5 sec dwell time after 24 hours of mixing can be classified into two classes based on the resistance to the flow. Class I materials are high resistant to flow when R≤1.1 and class II materials are low resistance to flow when 1.1<R<1.75. Increasing the P/L ratio decreased R in both CC and VG resulting from the decreased ethanol content. When stored dry only VG showed a decrease in R with time (more notable in VG1.5) which might be due to higher amount of ethanol evaporating from the material whereas all other materials had less or no ethanol so leading to increase in R. When stored in DW and AS all materials showed a decrease in flow because of leaching of plasticiser and uptake of water. The trends seen in R of materials in different immersion solutions are similar to the ones seen in CCR.

Ethanol content has been found to be one of the major factors that influence flow of tissue conditioners in a number of studies. Jepson *et al.* (2000) studied the viscoelastic properties of different commercial tissue conditioners including VG and CC in different immersion solutions including DW. There was a reduction in creep compliance in all materials with time although to different degrees depending on immersion solution. However it was noted that the formulations with higher ethanol content had higher reductions. This result agrees with the findings of the present study where VG and EPLS having higher ethanol content showed decrease in CCR. Murata *et al.* (2010) investigated the viscoelastic behaviour of various commercial home reliners (containing polyvinyl acetate and ethanol) for 7 days and found that these have no elastic component and behaved only in a viscous manner i.e. very high flow (CCR). The authors attributed the higher ethanol content of 20-30% in these material as the leading factor of this finding compared to the tissue

conditioner's (where the ethanol content is much less) in their earlier study which also showed elastic response (Murata *et al.*, 2001b; Murata *et al.*, 2010). It is believed that more ethanol will result in greater plasticiser loss (Jones *et al.*, 1988; Liao *et al.*, 2012), as explained in section 5.4, resulting in reduced flow due to reduced polymer chain mobility and so reducing CCR.

For the P/L materials, the addition of CHD and NaF decreased CCR at 1 hour (Table 4.13), the additives acting as filler particles to reduce flow. However, their addition had little effect on the CCR of EPGS throughout the 1 week study when stored dry. When stored dry, the additives generally increased the CCR of VG and EPLS at 24 hours and 1 week with the exception of the NaF containing EPLS formulations where there was a slight decrease, it is not clear what the cause of this effect was. Stored in DW and AS the additives had a variable effect depending on the material, however changes were low.

At the end of the 1 week study VG and EPLS formulations in all storage conditions had similar CCR values whereas those for EPGS were slightly lower but were more stable throughout the 1 week study.

For all materials, addition of CHD and NaF had a variable effect on R (Table 4.14) whereas in CCR addition of additives had little or no effect. When stored dry again with exception VG, which has the highest ethanol content, R generally increased with time from 24 hrs to 1 week. When stored in DW and in AS all materials showed a decrease (or remained unchanged) in R which, as explained earlier is because of the leaching of the plasticiser and water uptake by the material. In DW the decrease

in R was more than in AS owing to the fact that AS contains more variables and with the addition of additives makes the whole water uptake and leaching of constituents more complex (Ali, 2010). In general the CCR and R in DW follows the same trends but the CCR had more variable effects in AS.

Variabilities in hardness, penetration ratio and CCR measurements of formulations containing additives may have been affected by their distribution in the matrix. Differences could have been caused by composition, especially ethanol content where the solubility of CHD could improve homogeneity in the higher ethanol materials.

It was not possible to compare the effect of additives on flow (CCR) with other studies as none were available in current literature.

5.8 Summary

When developing a new tissue conditioner formulations it is important to know basic properties that define its use. These include gelation time, water uptake behaviour, hardness and creep compliance (flow properties). Gelation time is important to know as they are chairside materials and it is desirable to have a short gelation time however with a pre-gelled system this is not important. Tissue conditioners are used in an aqueous environment so it is essential to study their water uptake behaviour. Similarly hardness and creep are important to know as they define the materials usage in a particular clinical situation.

As discussed above the gelation time for pre-gelled system is not important but for P/L systems the gelation time with and without the additives were not ideal but acceptable.

When studying the water uptake behaviour of these materials % weight changes are measured which also reflects the dimensional changes occurring in them. So weight change of ~8.8% in EPLS 9%CHD (lowest among all formulations) and ~47.6% in EPLS 9%CHD (highest among all formulations) after 4 weeks mighty be problematic in its use in mouth. Higher the % weight change reflects higher dimensional change so the fitting of the denture might be compromised if it is used for periods of 4 weeks even after 1 week the % weight changes range from ~4% to ~25% which is still too high to be acceptable for clinical use. So they must be replaced after every 2-3 days to have optimum properties (Braden *et al.*, 1997).

CHD is a good anti-microbial and anti-fungal agent to be added in these materials as they can also be used in denture wearers to improve the general oral health (Petersen and Yamamoto, 2005; Sloane *et al.*, 2013), rather than only in patients with candidal infections. As discussed above the amount of CHD in the formulation affects the % weight change. Increasing the % of CHD increases the water uptake and addition of NaF further enhances the % weight change which will all contribute to the problems of the fitting of the denture over period of 1 week and more.

Hardness and CCR values are important for the functional classification of the materials in its intended use. There are no ideal values found in the literature for the hardness of tissue conditioners, however Craig (1997) suggested that Shore A

hardness of 13 to 49 in 24 hours will not interfere with the use of tissue conditioners in mouth. According to Gonzalez (1977) the ideal Shore A hardness for temporary denture liners should be between 20 and 25 without changes during use. The results in this study seem to satisfy both the author's criteria. Similarly the penetration ratios (R) of the materials were in the range of class II low resistance to flow which also satisfies the ISO 10139-2 standards. The additives did not have a sufficient effect on the hardness and CCR values that would hinder function, however the EPGS formulations had the most stable CCR values over the period of 1 week.

EPLS formulations are best used as vehicle of delivery of CHD as the increase in amount from 1% to 9% did not effected the % weight change of the materials but the addition of NaF increased the release and % weight change. Considering hardness and CCR, the NaF containing EPLS formulations had higher hardiness and lower CCR than the formulations without them. These formulations might be more useful as tissue conditioners rather than temporary lining material or functional impression material.

Based on all above properties EPGS would be best used as a tissue conditioner or as a temporary lining material although when additives are added the CHD release is not as high as compared to the other formulations.

CHAPTER SIX: CONCLUSIONS

6 Conclusions

- An ethanol-free, citrate-based, pre-gelled material, EPGS, has been developed with suitable physical properties to function as a tissue conditioner. It has been shown to have stable Shore A hardness values over 18 months storage, if refrigerated. Based on Shore A hardness and flow (CCR) EPGS is best suited to be used as temporary soft lining materials or as tissue conditioner.
- The P/L citrate-based material, EPLS had comparative or improved properties compared to the commercial P/L tissue conditioners. Based on the Shore A hardness and CCR. EPLS is best suited as a tissue conditioner.
- The ethanol content of tissue conditioners was found to play a key role affecting all properties.
- Addition of CHD and NaF affected the properties of both commercial and experimental tissue conditioners; these effects varied with composition. Their addition increased both water uptake and solubility which in turn may affect their dimensional stability. However they did not have sufficient effect on the Shore A hardness and CCR values to affect the function of the materials; EPGS formulations presented with more stable values.
- All materials, both commercial and experimental, released CHD, but at different levels. Addition of NaF further increased the amount of CHD released.
- Generally all water uptakes plots against $t^{½}$ appeared linear (Fickian uptake kinetics). It can be concluded that in the presence of sodium fluoride, the water uptake process of EPGS became complex and was anomalous.
- Water desorption was rapid and Fickian in nature for all systems. D_{des} values increased with increasing CHD content in formulations from 1% to 9% (except for EPLS), and when NaF was added (except VG 9%CHD). D_{des} was slower for VG (10^{-11} m^2sec^{-1}) reflecting the higher ethanol content in this formulation.

- CHD was released in two phases. Initially, it was released by a Fickian process from VG 1%CHD, VG9%CHD, EPLS 1% CHD, EPLS 9%CHD+F. For all other formulations, the initial and second phases of release were by non Fickian kinetics.
- It proved difficult to analyse the process by which fluoride ions were released. Therefore it can be concluded that fluoride was released by an anomalous process.
- The replacement of BPBG (VG old) with a citrate-based plasticiser in VG has changed its water uptake profile dramatically, with the current VG showing continued weight loss on storage in DW. Thus it can be assumed that the citrate-based plasticiser leaches out more readily than BPBG. This highlights the importance of reducing the ethanol content in the experimental ATBC-based materials to reduce plasticiser leaching, as was shown in this study for EPLS and EPGS.
- Use of Shore A hardness measurements using different dwell times has proved a useful method for assessing CCR (flow properties) of tissue conditioners, particularly change over time when stored in various conditions. As noted in the discussion section, findings from this study were in general agreement with other studies where different methods were used.

CHAPTER SEVEN: FUTURE WORK

7 Future Work

- Further development of EPGS is required in terms of its clinical chairside delivery. Home use materials are usually in the form of a thin sheet or contained in a tube, so these formats should be initially studied.
- Water uptake behaviour in other liquids such as artificial saliva and food simulating liquids should be studied to help evaluate *in vivo* performance. Additionally interaction with commonly used denture cleansers needs to be assessed.
- Plasticiser and ethanol leaching needs to be quantified using such methods as UV/Vis spectroscopy or high performance liquid chromatography (HPLC).
- Although all the materials did release CHD, microbiological studies should be carried out to assess whether they are effective against C*andida albicans.*
- The amount of CHD used in the formulations needs to be optimized by using different concentrations so that maximum percentage release can be obtained.
- Release of other drugs from this material should be studied to investigate their potential for use in the treatment of other oral infections e.g. topical delivery of steroids for the treatment of lichen planus.
- CCR results should be compared with other testing methods for viscoelasticity like creep test or stress relaxation test so that a better understanding could be obtained about the accuracy of results obtained by the methodology used in this study.
- Release of F from the materials and its role in drug release needs to be further explored.

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Appendix

A1. Ethanol Content

To measure the ethanol content of Visco-gel (VG) liquid a glass jar of 100ml capacity was weighed using an AE Mettler balance (Metler – Toledo Ltd, Leicester, UK) accurate to four decimal places. 10 g of the VG liquid was weighed in the jar and the jar was left on a bench top for 24 hours so that the ethanol in the liquid could evaporate in air. After 24 hours the jar was reweighed and the difference of weight was found to be 6.2% w/v.

As a control the liquid used in experimental powder liquid system (EPLS) was used. This liquid contained 5ml ethanol and 95ml of Acetyl tributyl citrate (ATBC). When 10g of this liquid was weighed after 24 hours the difference between the two readings were 3.5% w/v.

From these two readings it can be concluded that the VG liquid contains almost the double the amount of ethanol compared to the control liquid in EPLS.

A2. Difference between VG new and VG old

The manufacturers of VG changed the plasticiser used in its liquid from BPBG to a citrate based one. To investigate the effect of this plasticiser change on the properties of VG a study was conducted measuring particle size of the powders used, water uptake and desorption, Shore A hardness and creep compliance ratio (CCR) of both VG old and VG new were compared using the same techniques as mentioned in section 3.2.

Table 15 shows no significant difference was found between VG and VG Old polymer powder in terms of Mean particle size (D[v,0.5]), surface/volume mean diameter (D[3,2]) and volume mean diameter (D[4,3]).

Table 15 Mean of Mean particle size D[v,0.5], surface/volume mean diameter D[3,2] and volume mean diameter D[4,3] with SD of different polymer powders (n=5)

Powder	D[v,0.5]	SD (±)	D[3,2]	SD (±)	D[4,3]	SD (±)
VG old	32.54[a]	0.17	13.70[a]	0.54	34.16[a]	0.17
VG new	32.91[a]	0.42	14.31[ab]	0.28	34.67[a]	0.34

No significant difference ($p \leq 0.05$) between groups with same letters

The water uptake study of VG New and VG Old was carried for 12 weeks and the results are shown in Figure 4.9. Both VG Old 1.3 and 1.8 showed a similar water uptake profile of weight loss till day ~5 ($t^{1/2}$ =587.9) where they started to gain weight at different rates i.e. VG Old 1.8 gained weight more rapidly than VG Old 1.3 up to the end of experimental time period. VG Old 1.8 gained more weight (9.1%±0.9)

compared to VG Old 1.3 (1.3%±0.2). Both VG New 1.5 and 1.8 lost weight rapidly up to ~34 days ($t^{1/2}$ =1610) followed by an increase in weight to a final weight change of -5.8%±0.6 and -1%±0.8 respectively. This initial part is indicative of loss of material.

The changes seen in both VG old and VG when the P/L ratio was increased is due to decreased ethanol content in the formulation thus resulting in a lower weight loss with the lower P/L ratios. The weight changes seen in uptake profiles of VG Old verses VG reflect the roles of the different plasticisers in the two formulations (BPBG verses ATBC). This will be further discussed in the discussion.

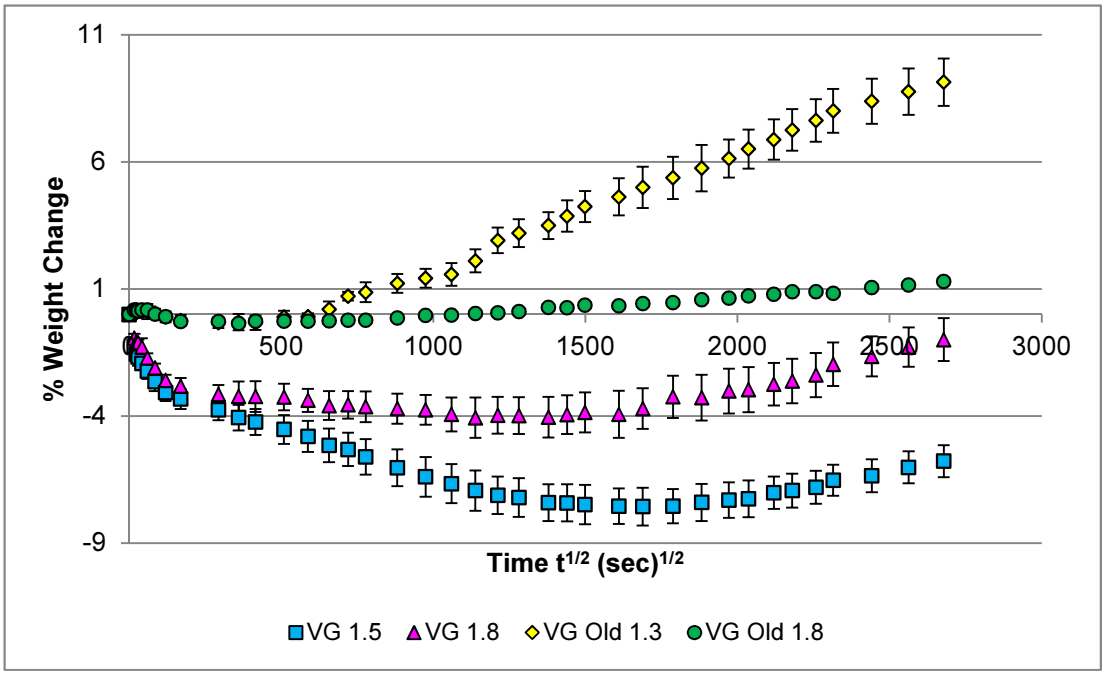


Figure 3: Mean (±SD; n=5) % weight change of VG New and VG Old formulations in DW at 37°C

Figure 4.20 showed the % weight loss of VG Old 1.3 and 1.8; VG New 1.5 and 1.8, on desorption where all reached equilibrium after 24 hours. The final weight loss for VG Old 1.3 was 9%±0.4 and fro VG Old 1.8 was 4.3%±0.2. VG New 1.8 and 1.8 reached equilibrium % weight loss of 15.6%±0.7 and 19.1±0.4 respectively.

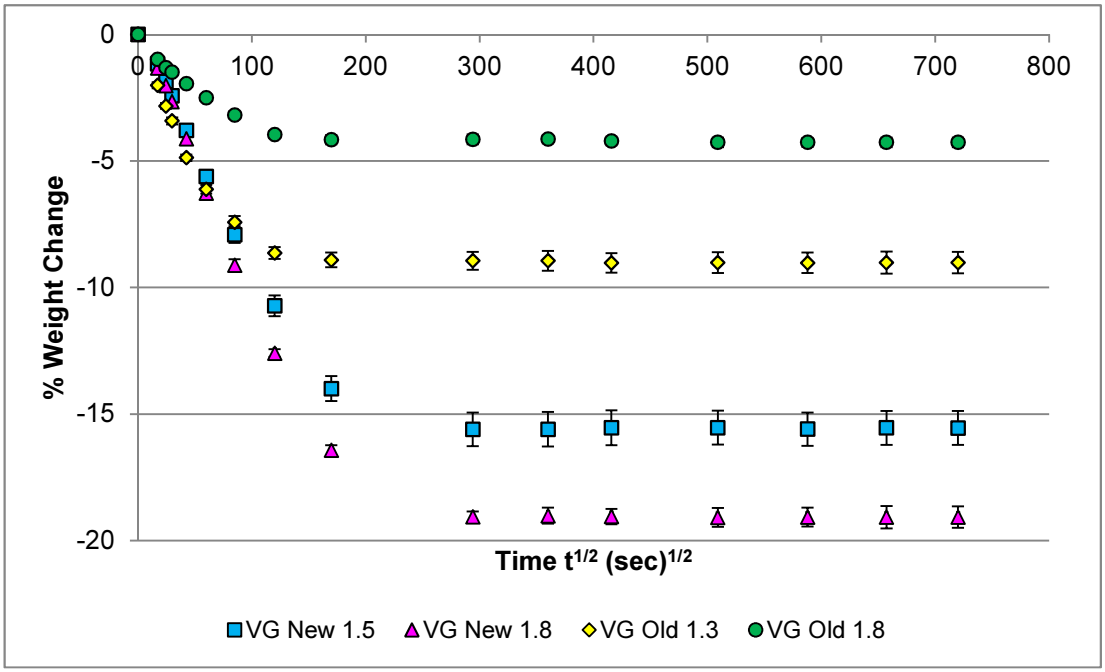


Figure 4: Mean (±SD; n=5) % weight loss of VG New and Old formulations in drying oven at 37°C

Table 4.3 showed the mean % weight change, % solubility, % real uptake and desorbed diffusion coefficient (D_{des}) data for the different tissue conditioner formulations. % weight change and % solubility increased when P/L ratio of VG New and Old was increased however % real uptake and D_{des} increased w=hen P/L ratio was increased in VG New and decreased in VG Old.

Table 16: Mean (±SD; n=5) % weight change, % solubility, % real uptake and D_{des} of VG New and Old formulations

Formulation	% Weight Change	% Solubility	% Real Uptake	D_{des} (m^2sec^{-1})
VG New 1.5	-5.7703	24.6 ± 1.6	18.8 ± 2.1	2.56×10^{-11}
VG New 1.8	-0.9896	25.4 ± 2.1	24.4 ± 2.6	1.96×10^{-11}
VG Old 1.3	9.1340	-4.3 ± 0.3	4.8 ± 0.4	1.03×10^{-10}
VG Old 1.8	1.2964	3.1 ± 0.1	4.4 ± 0.3	1.40×10^{-10}

Figure 5 shows the Shore A hardness of VG New and VG Old formulations at manufacturer's recommended and a higher P/L ratio 1 hour after mixing. Increasing the P/L ratio increased the hardness of the material. The Shore A hardness of VG was increased when the plasticiser was changed from BPBG to citrate based plasticiser.

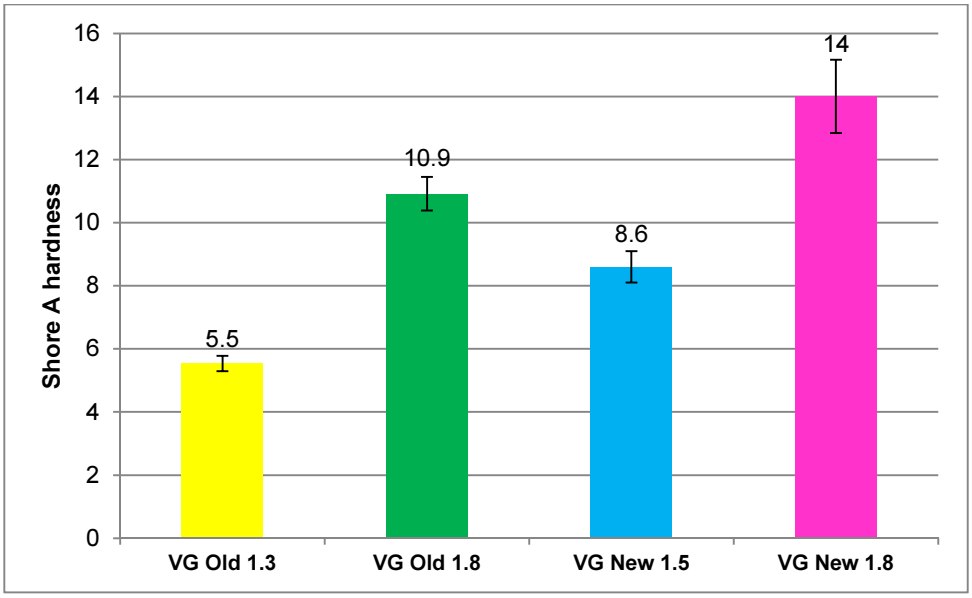


Figure 5: Mean (±SD; n=6) Shore A hardness of VG New and VG Old formulations at different P/L ratios, 1 hour after mixing

The Shore A hardness of the different VG formulations decreased with increasing dwell time at 1 hour after mixing (Figure 6). CCR of VG old increased with increasing the P/L ratio whereas it decreased in VG New as shown in Figure 7.

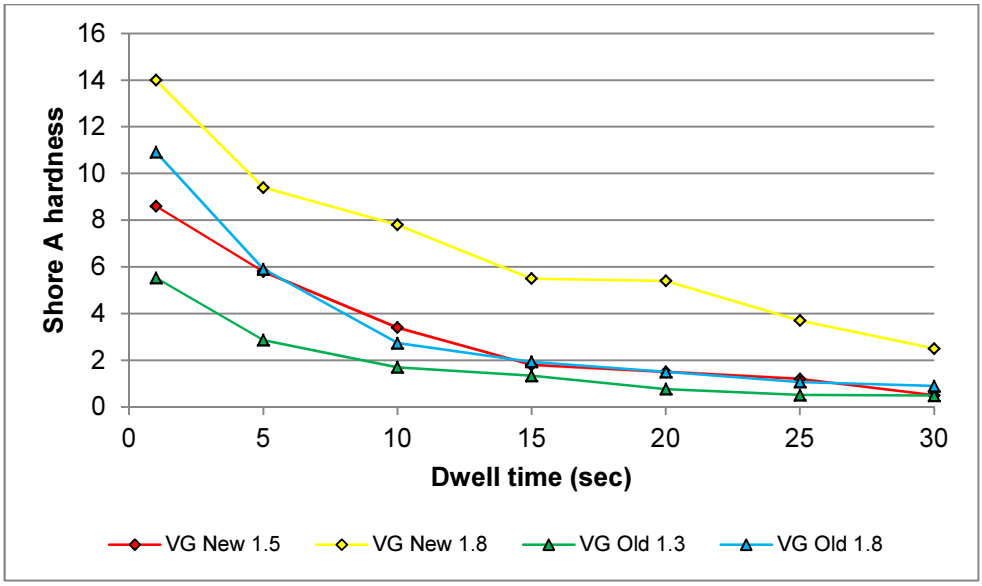


Figure 6: Mean Shore A hardness at different dwell times of VG New and VG Old formulations at different P/L ratios, 1 hour after mixing

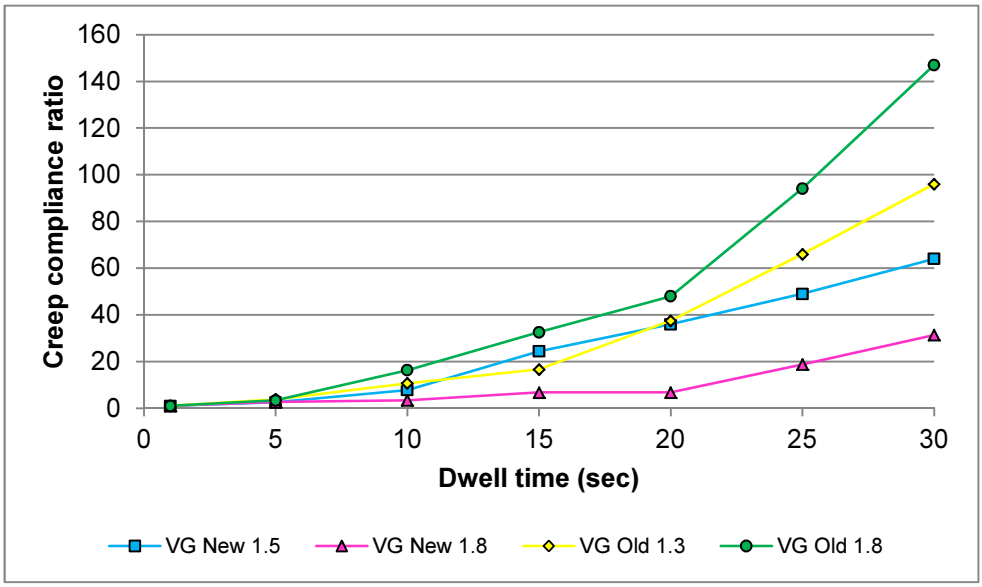
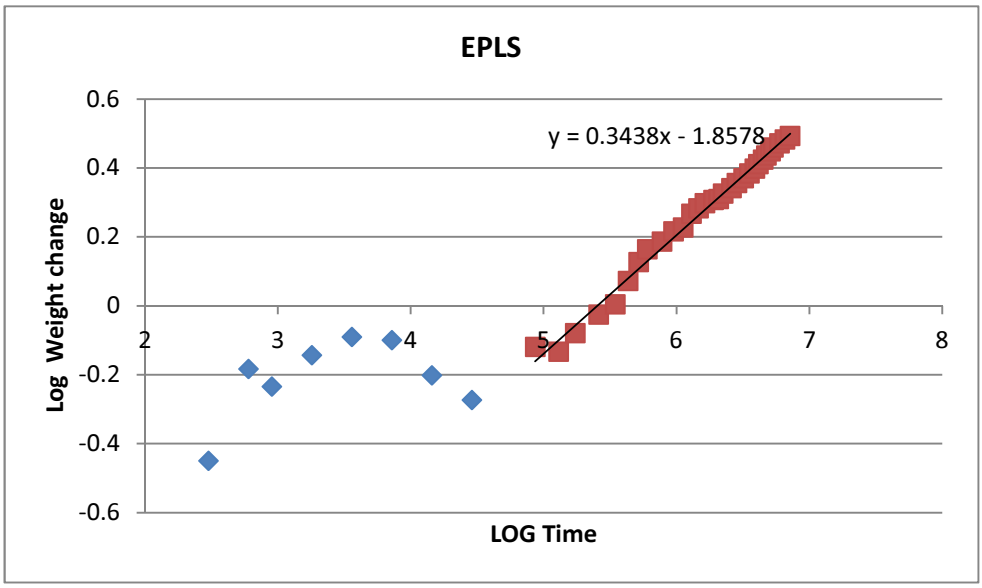
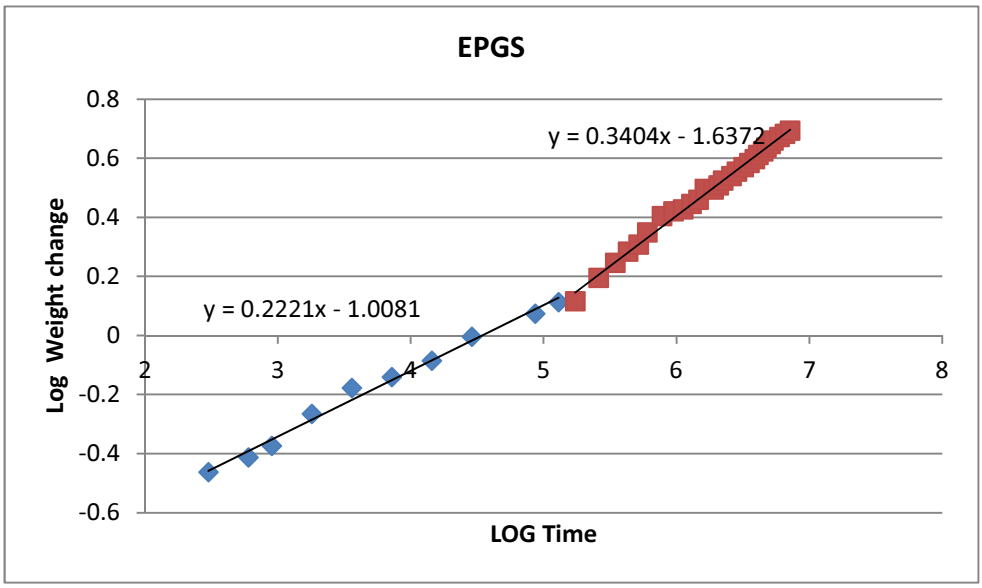


Figure 7: Mean CCR of VG New and VG Old formulations 1 hour after mixing

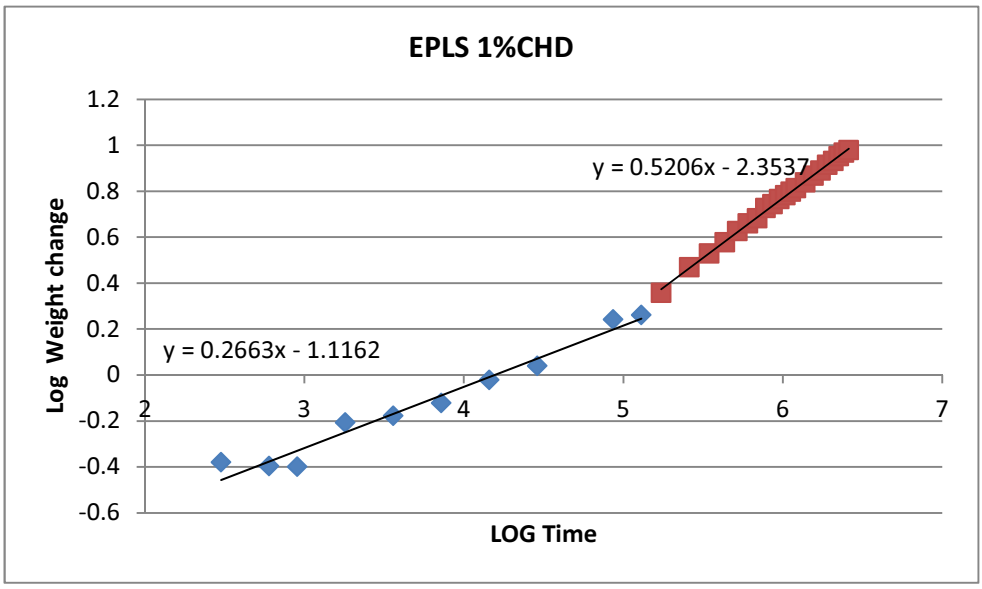
A3. Log-log graphs for water uptake studies



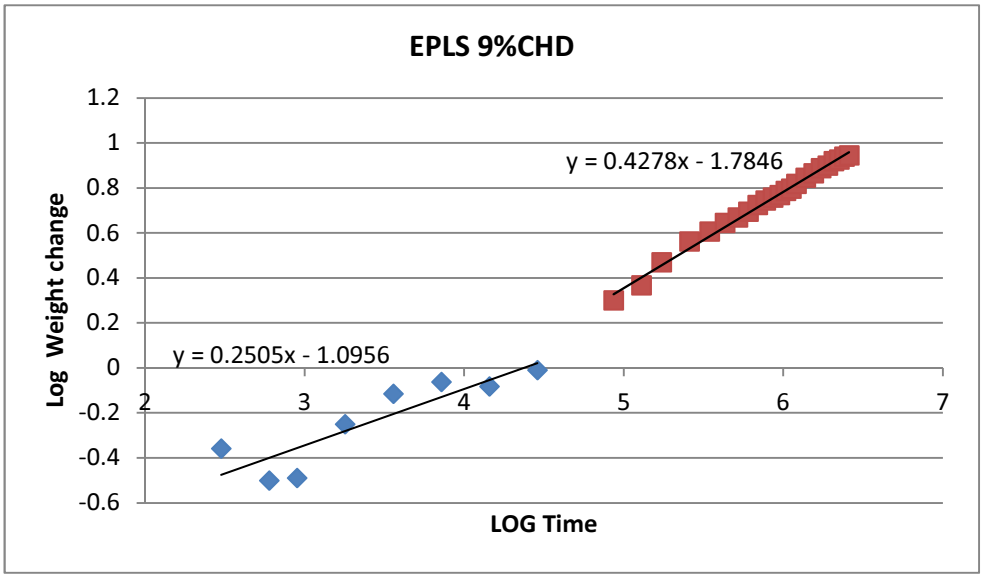
log of % weight change of EPLS against log time



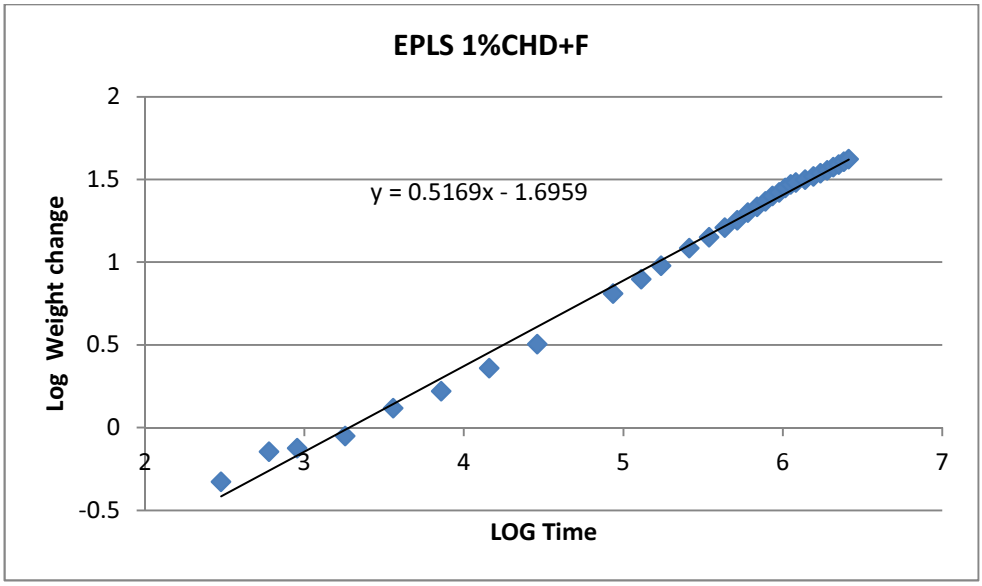
log of % weight change of EPGS against log time



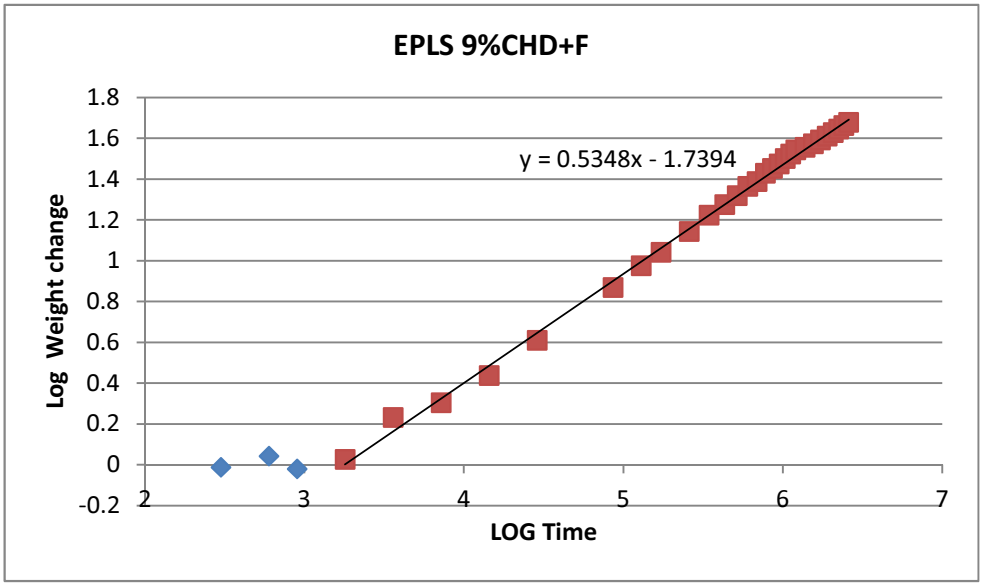
log of % weight change of EPLS 1%CHD against log time



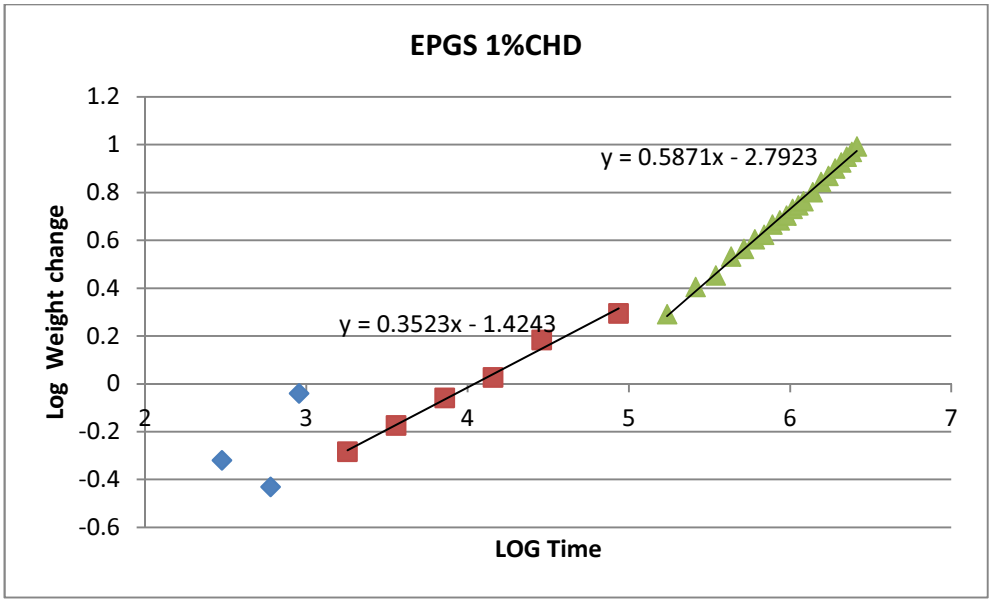
log of % weight change of EPLS 9%CHD against log time



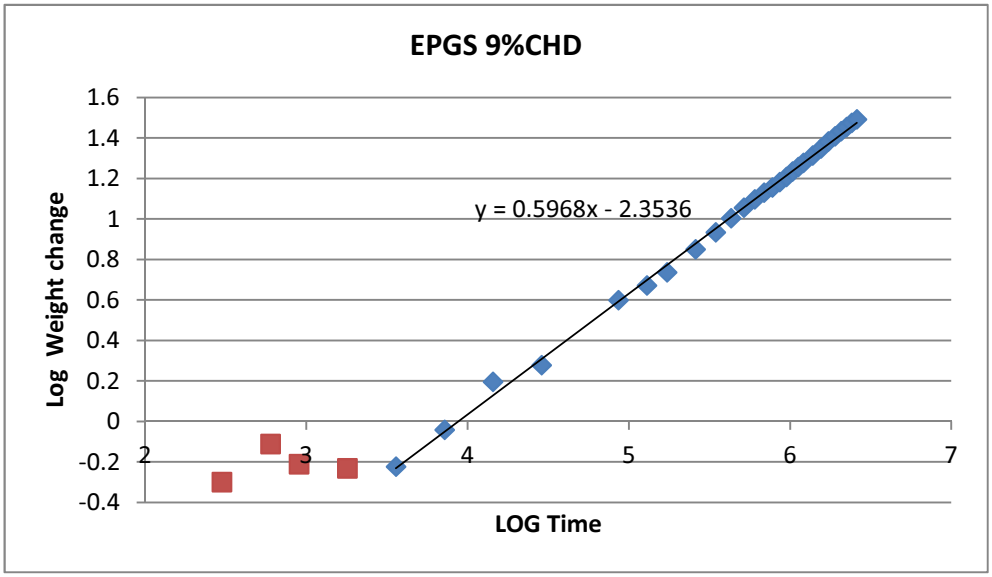
log of % weight change of EPLS 1%CHD+F against log time



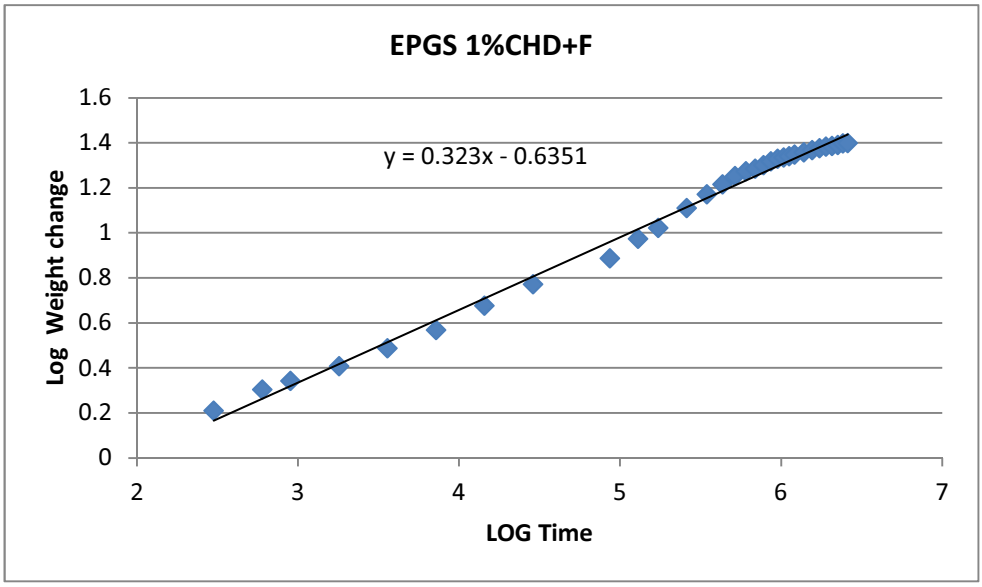
log of % weight change of EPLS 9%CHD+F against log time



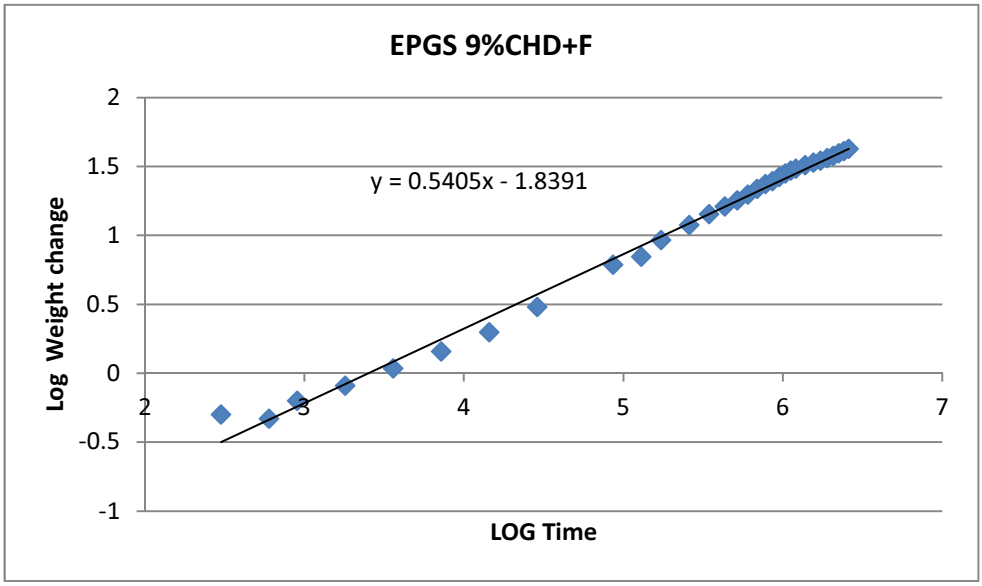
log of % weight change of EPGS 1%CHD against log time



log of % weight change of EPGS 9%CHD against log time

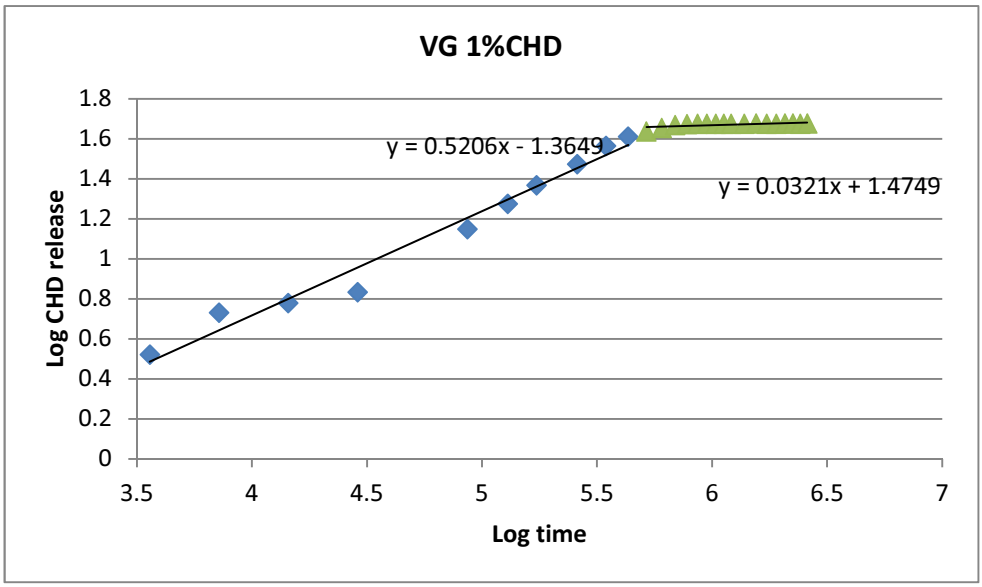


log of % weight change of EPGS 1%CHD+F against log time

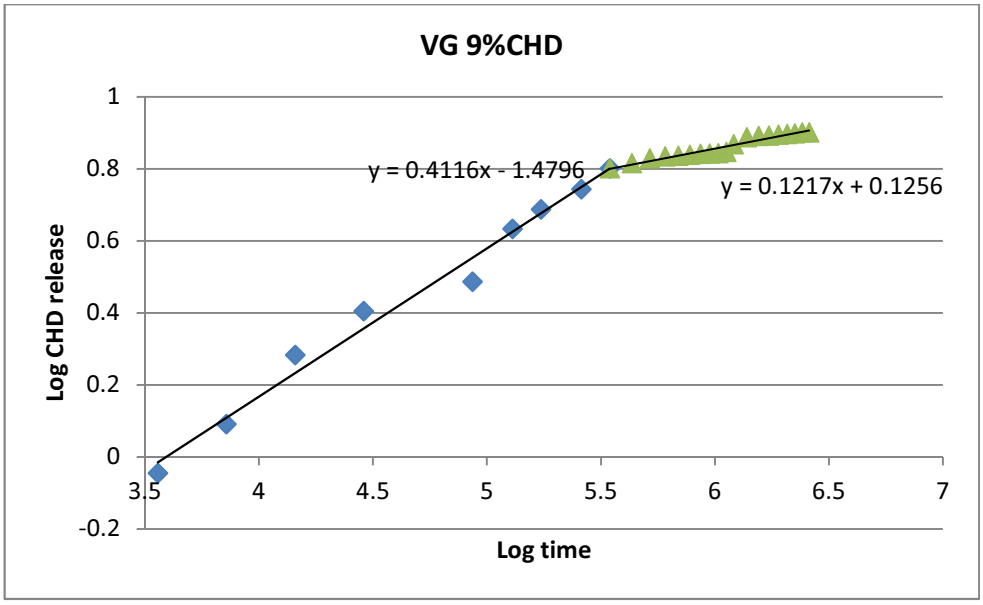


log of % weight change of EPGS 9%CHD+F against log time

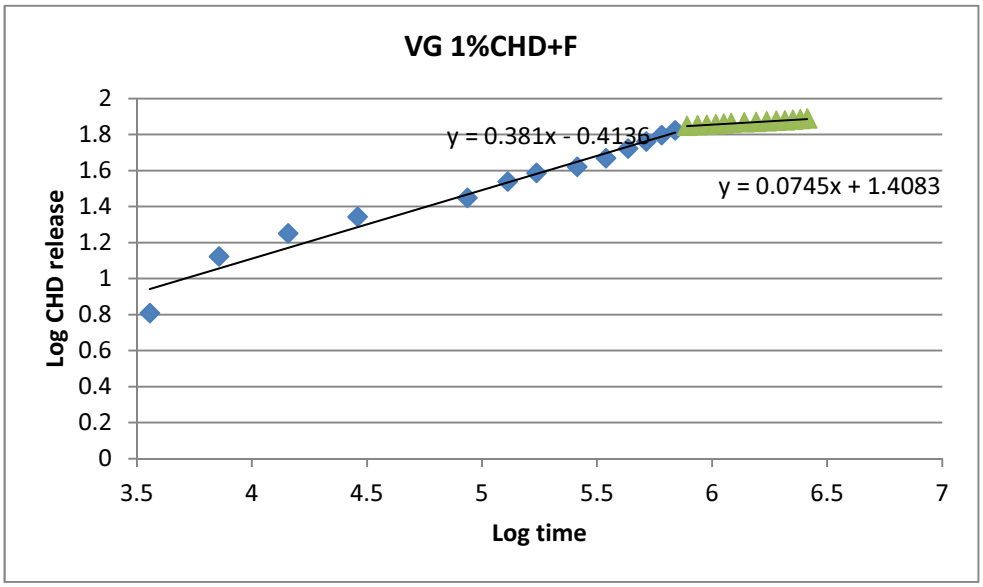
Chlorhexidine Release for 4 weeks



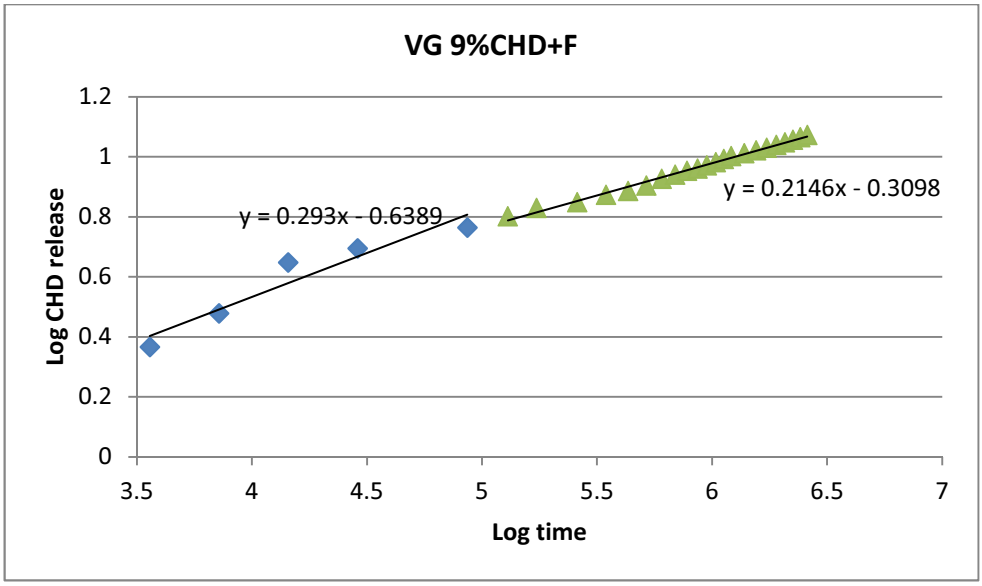
Log % CHD release of VG 1%CHD against log of time (sec)



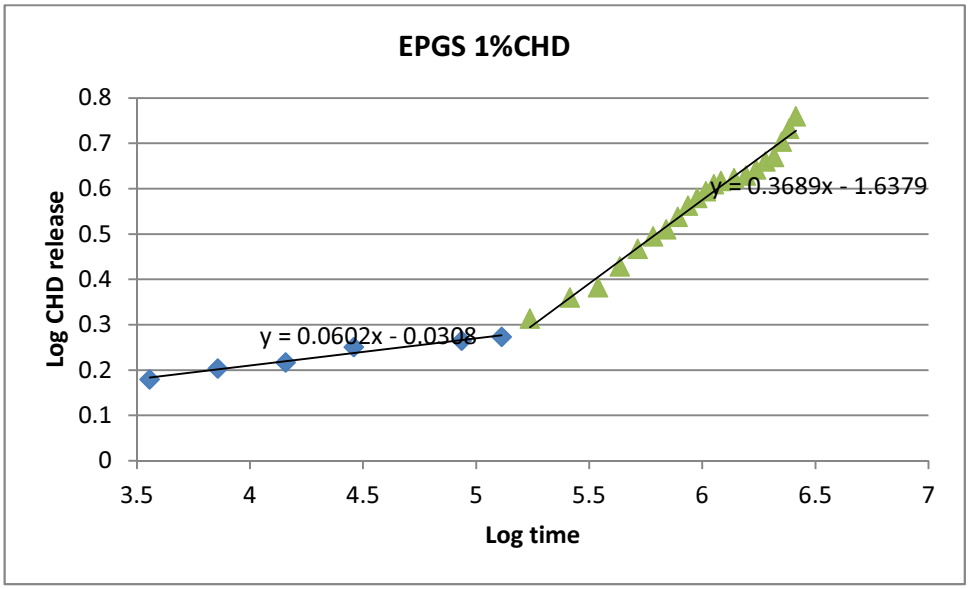
Log % CHD release of VG 9%CHD against log of time (sec)



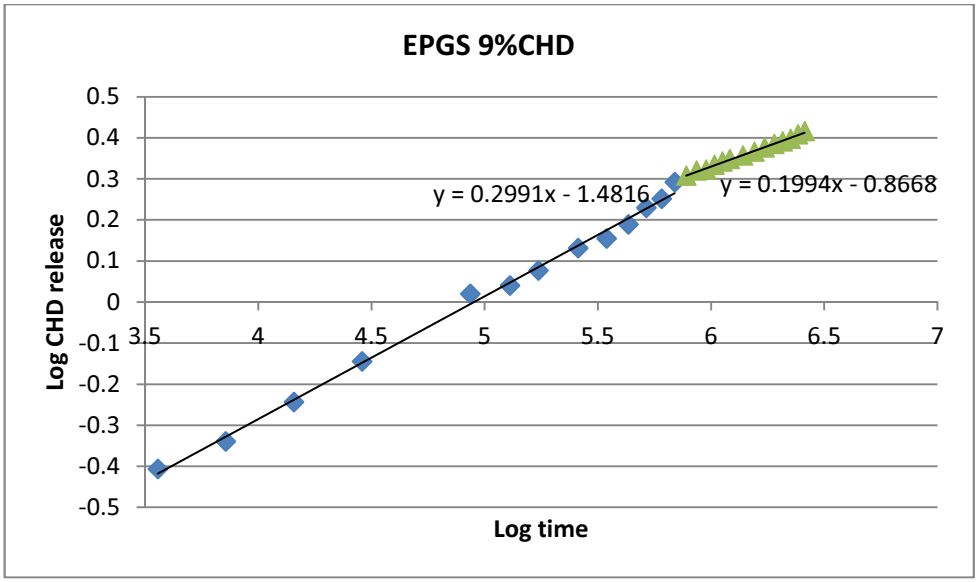
Log % CHD release of VG 1%CHD+F against log of time (sec)



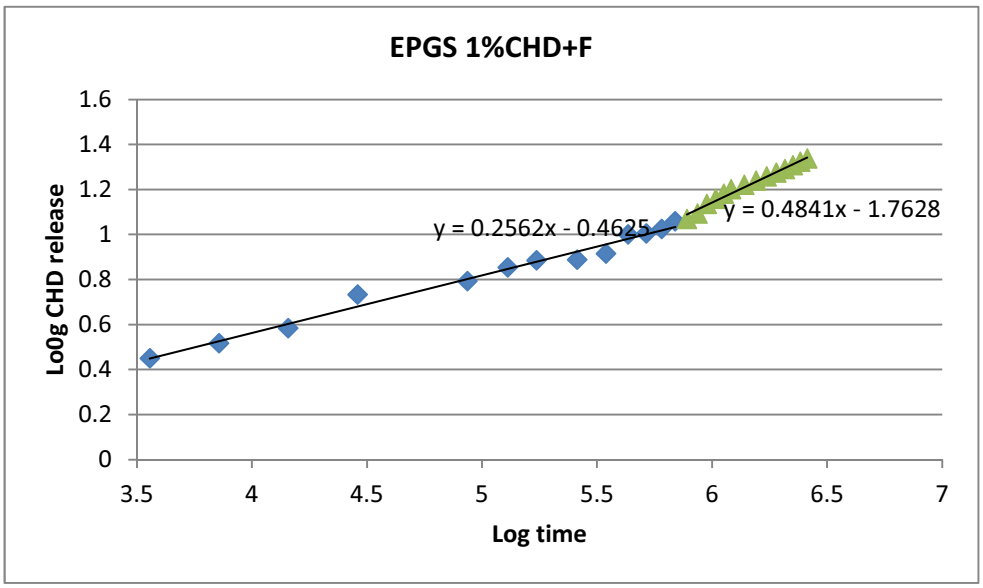
Log % CHD release of VG 9%CHD+F against log of time (sec)



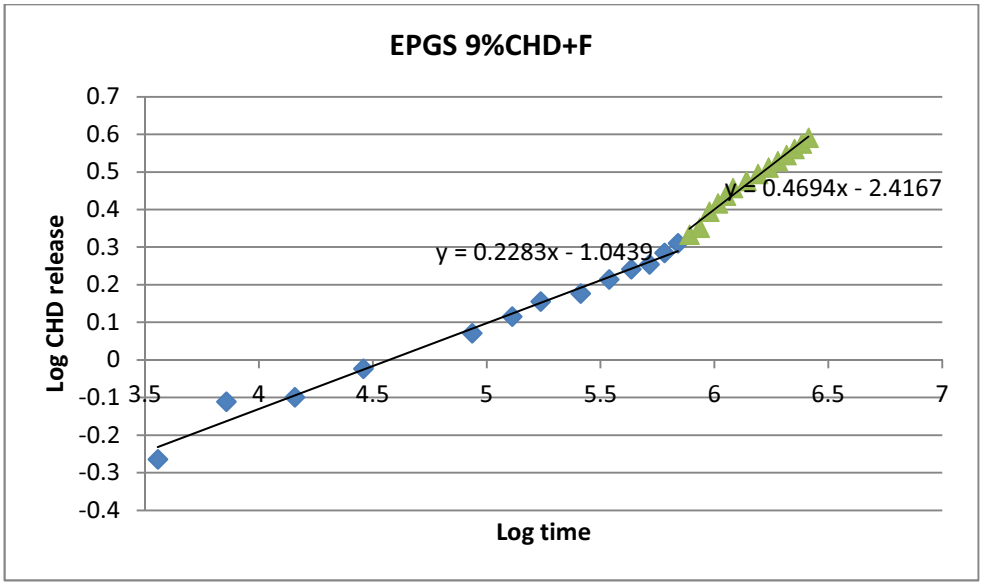
Log % CHD release of EPGS 1%CHD against log of time (sec)



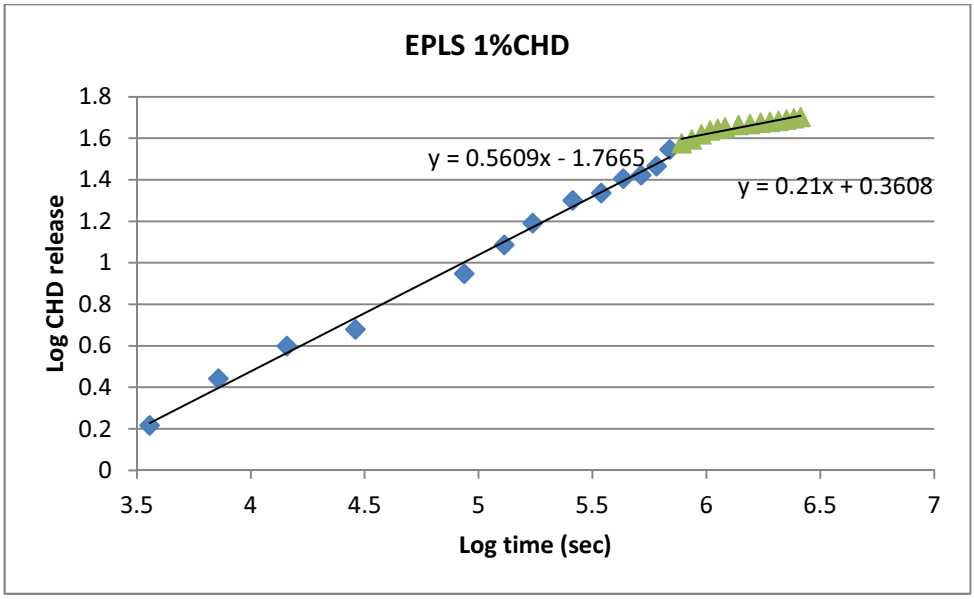
Log % CHD release of EPGS 9%CHD against log of time (sec)



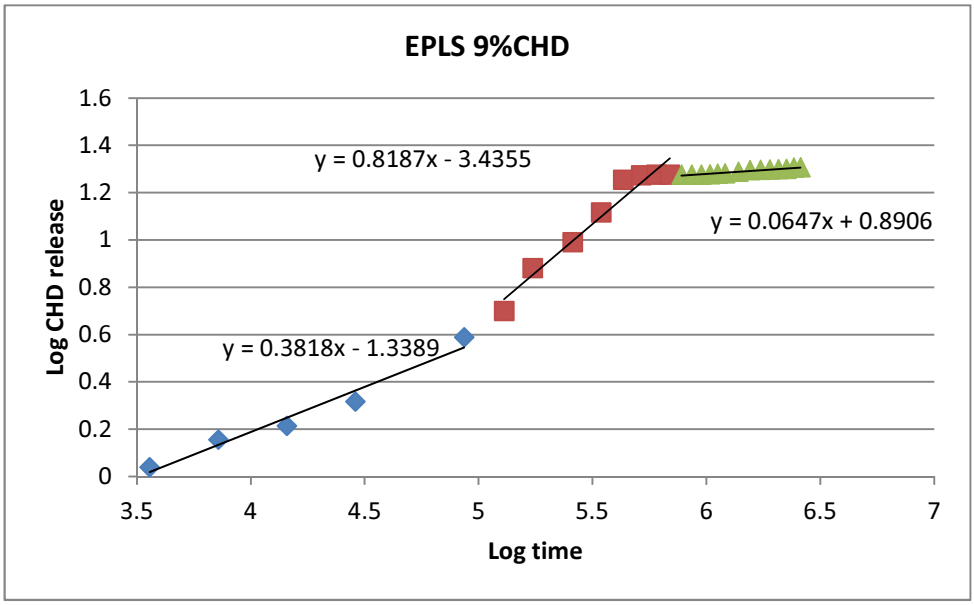
Log % CHD release of EPGS 1%CHD+F against log of time (sec)



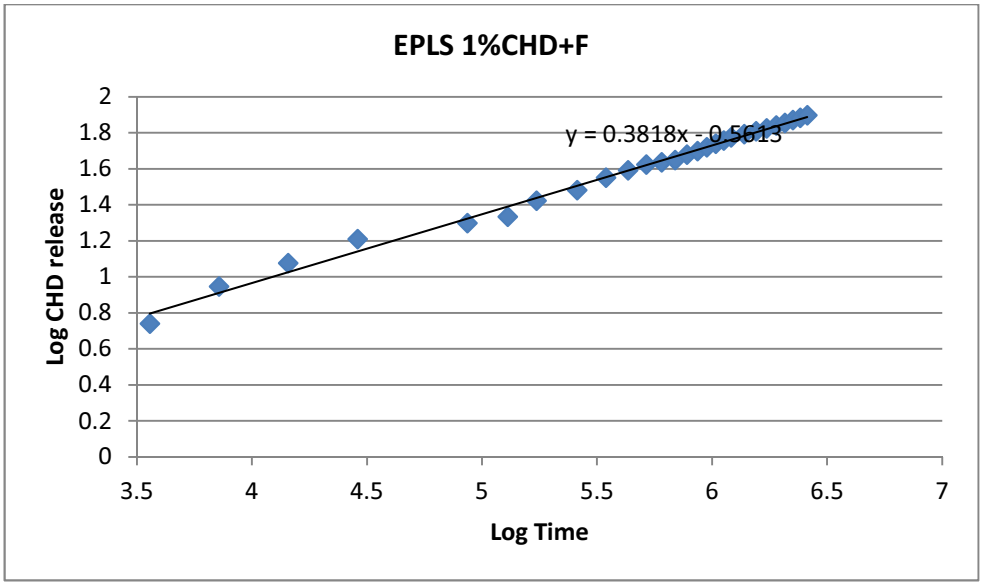
Log % CHD release of EPGS 9%CHD+F against log of time (sec)



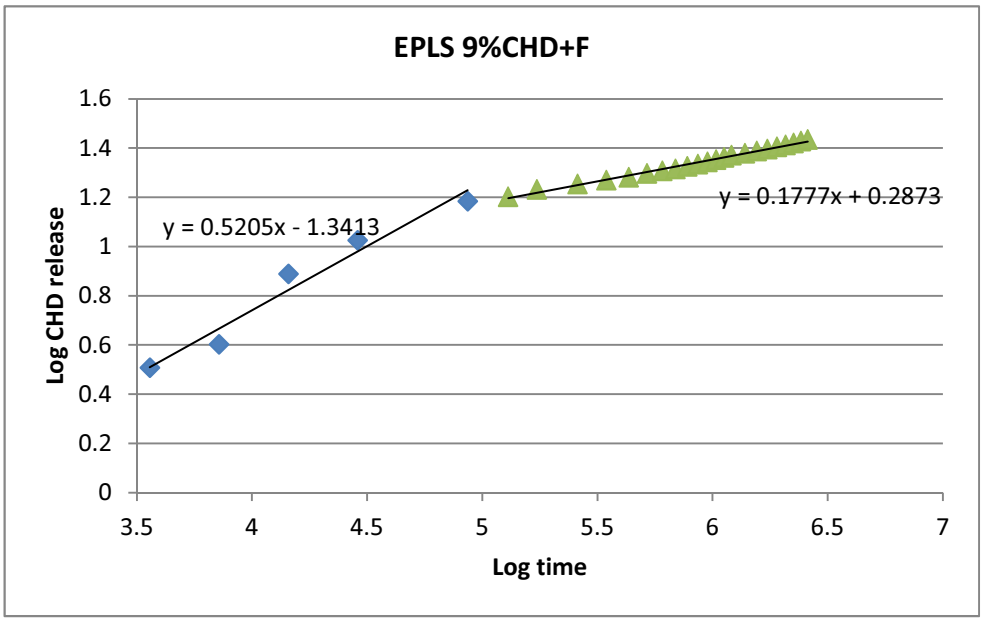
Log % CHD release of EPLS 1%CHD against log of time (sec)



Log % CHD release of EPLS 9%CHD against log of time (sec)

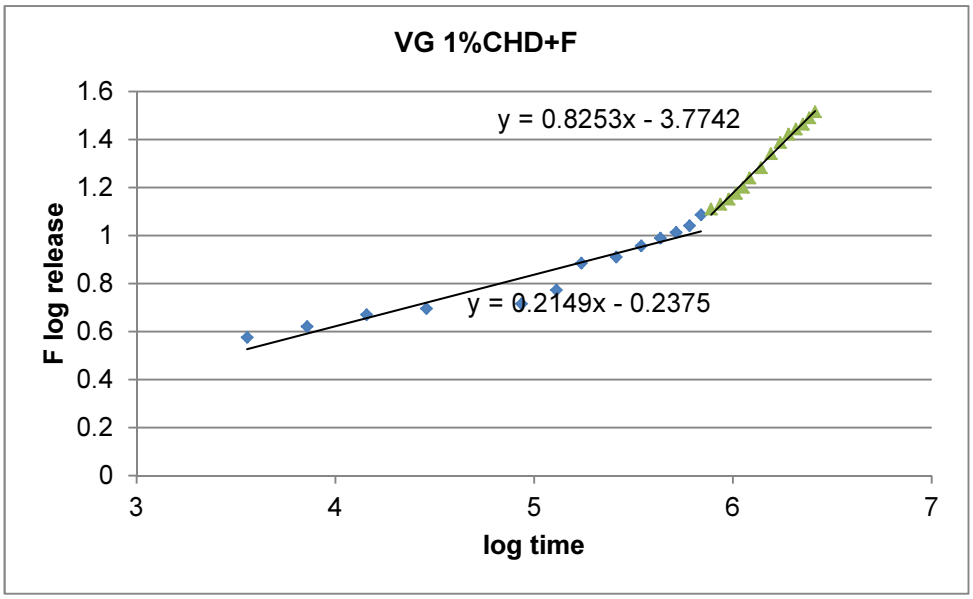


Log % CHD release of EPLS 1%CHD+F against log of time (sec)

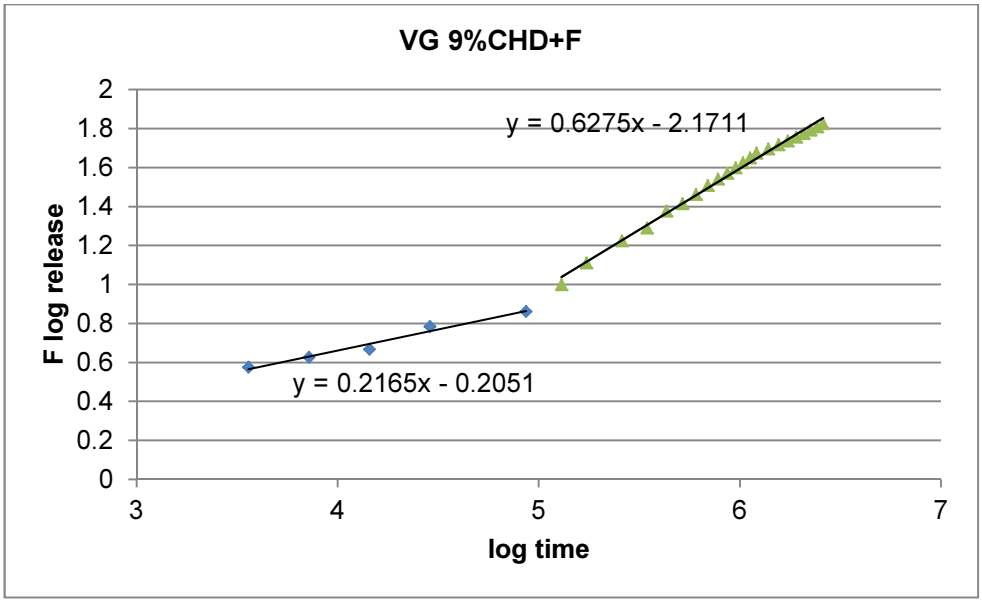


Log % CHD release of EPLS 9%CHD+F against log of time (sec)

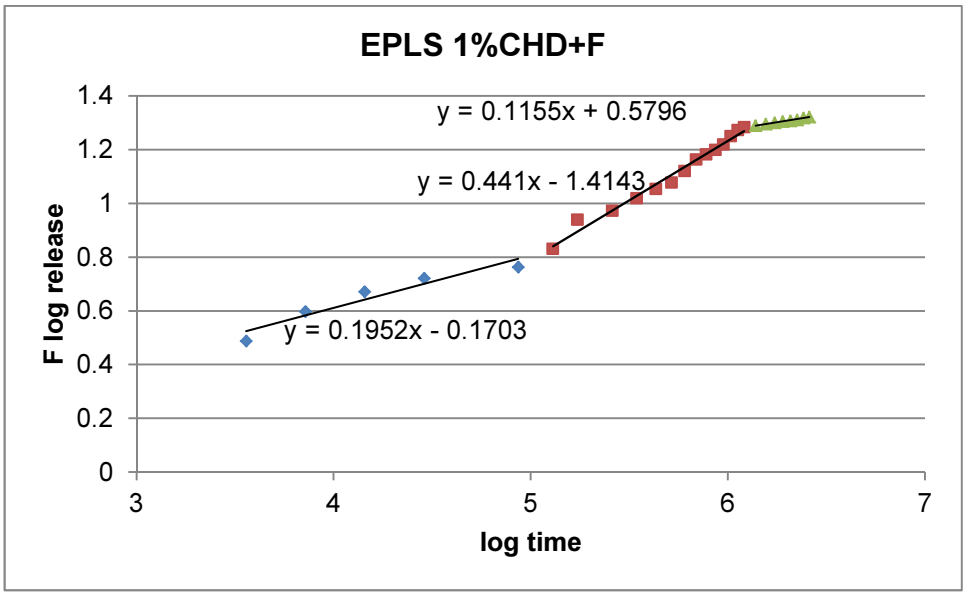
Fluoride Release for 4 weeks



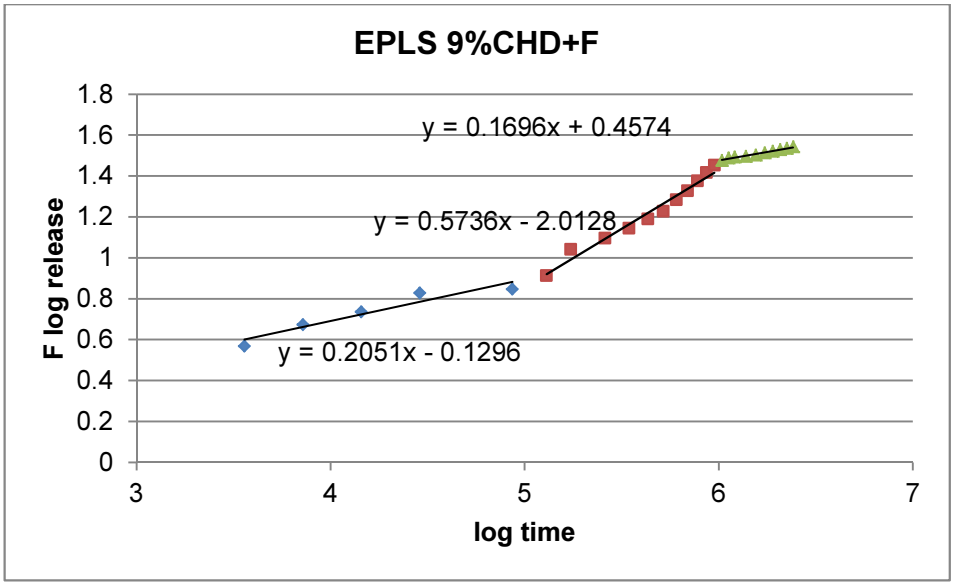
Log % F release of VG 1%CHD+F against log of time (sec)



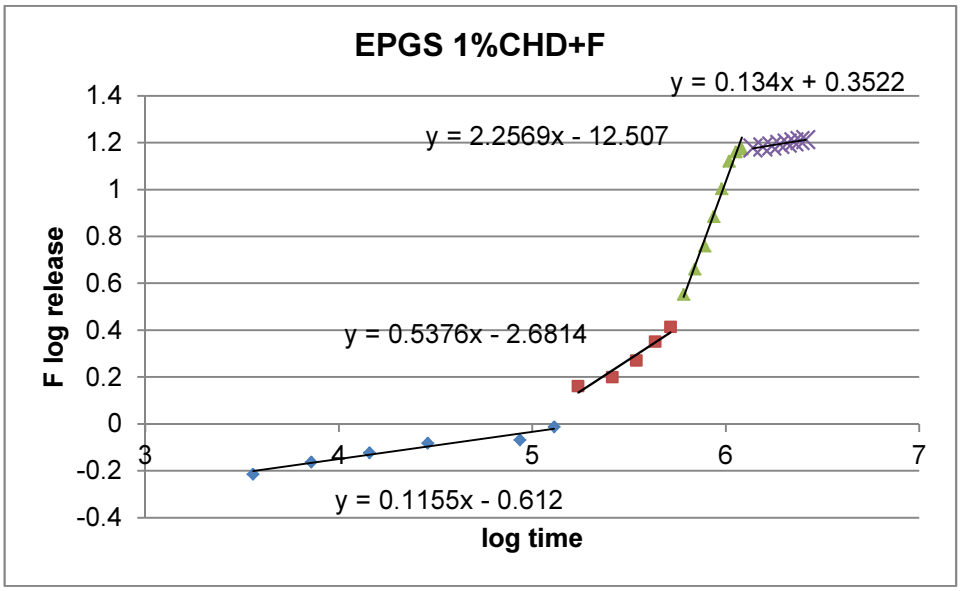
Log % F release of VG 9%CHD+F against log of time (sec)



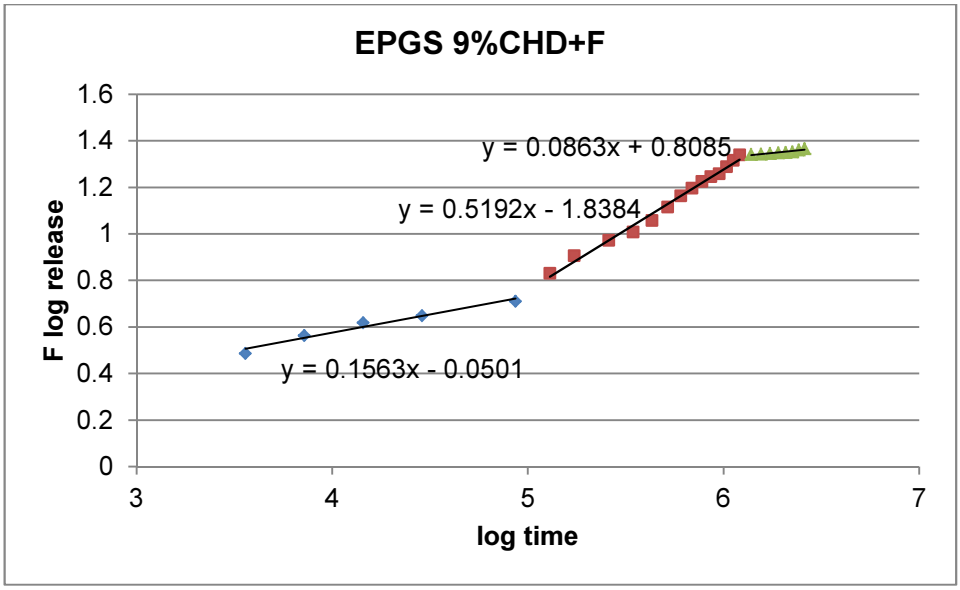
Log % F release of EPLS 1%CHD+F against log of time (sec)



Log % F release of EPLS 9%CHD+F against log of time (sec)

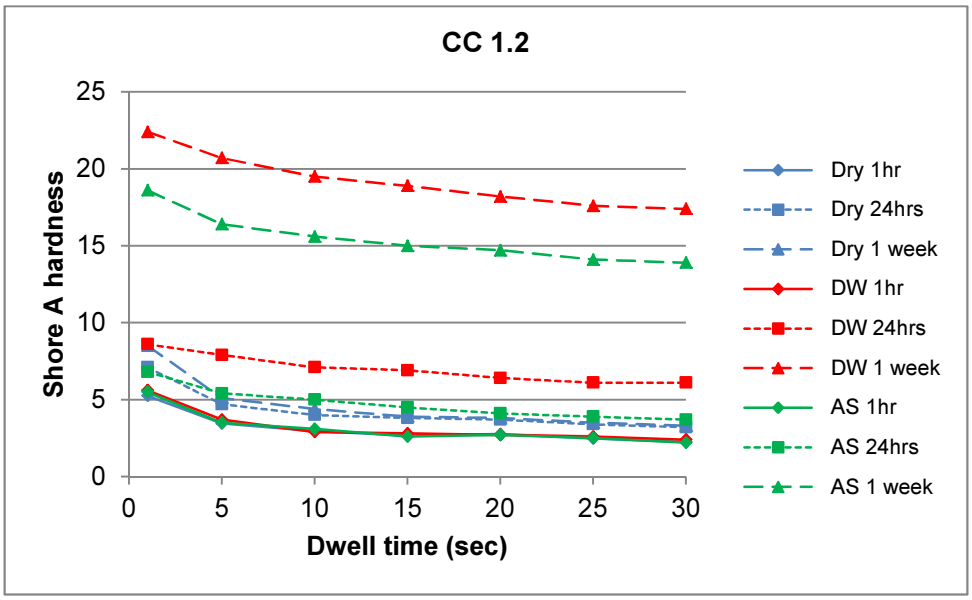


Log % F release of EPGS 1%CHD+F against log of time (sec)

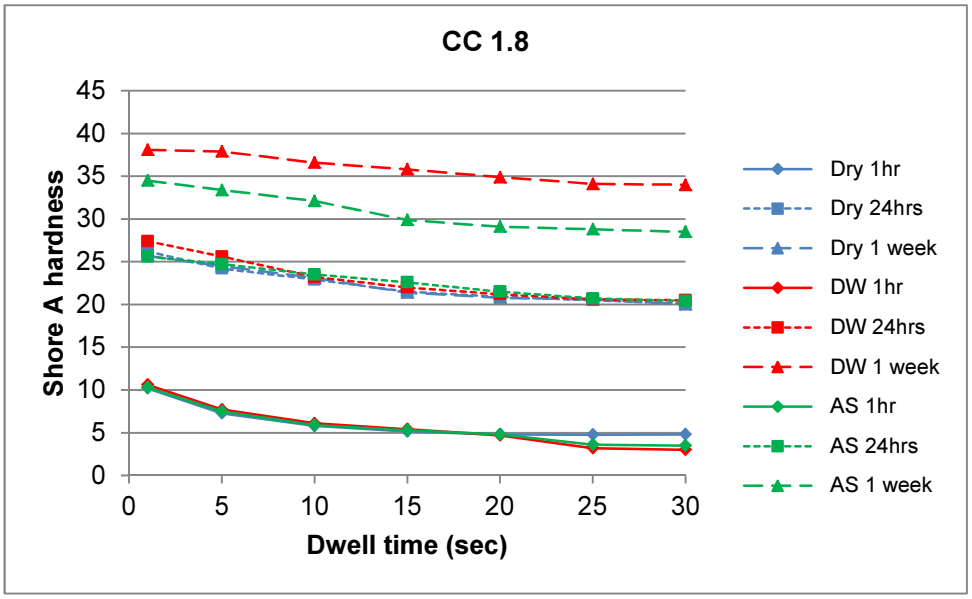


Log % F release of EPGS 9%CHD+F against log of time (sec)

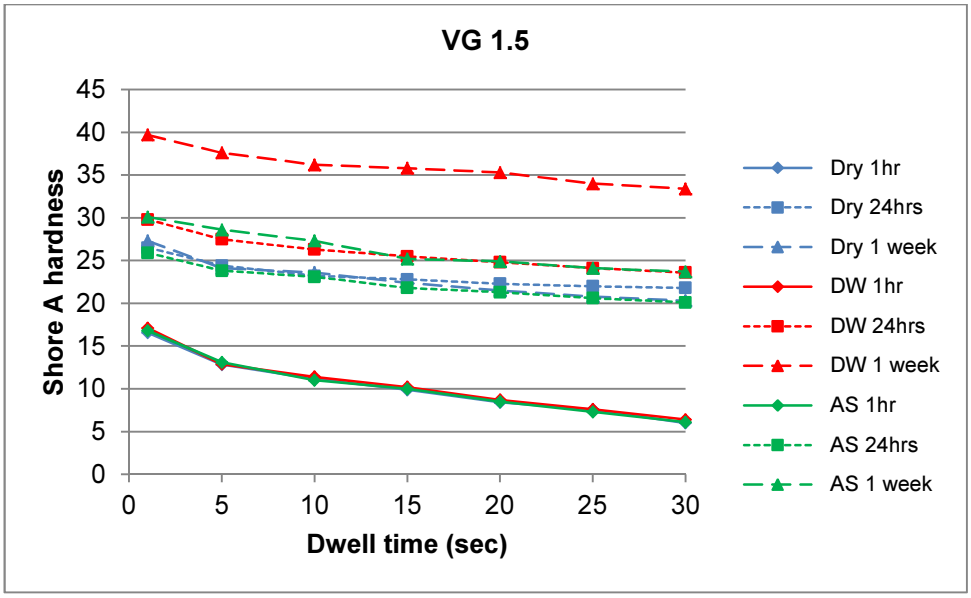
A3. Shore A hardness



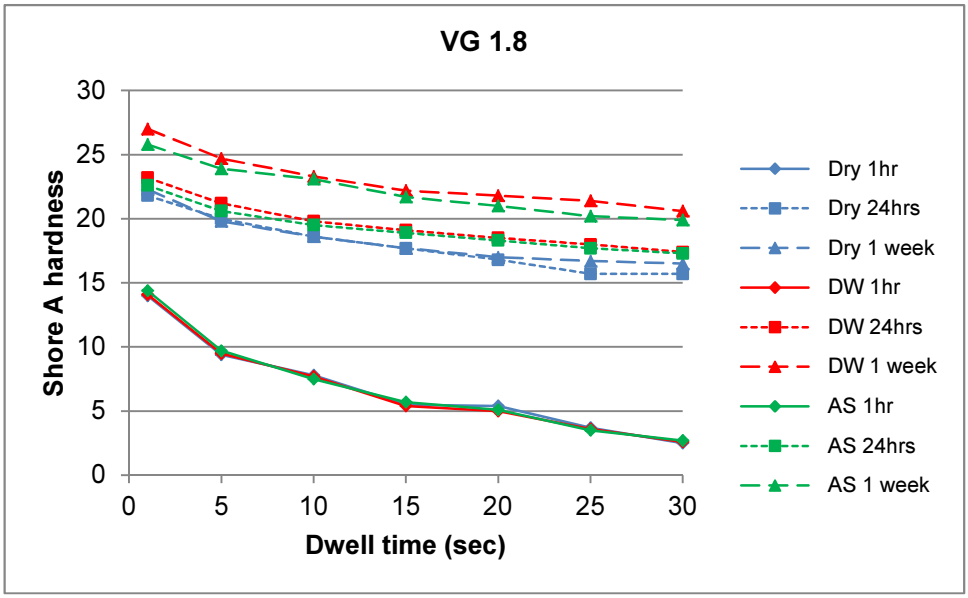
Mean (n=6) shore A hardness of CC 1.2 at different dwell times stored at 37°C in dry, DW and AS



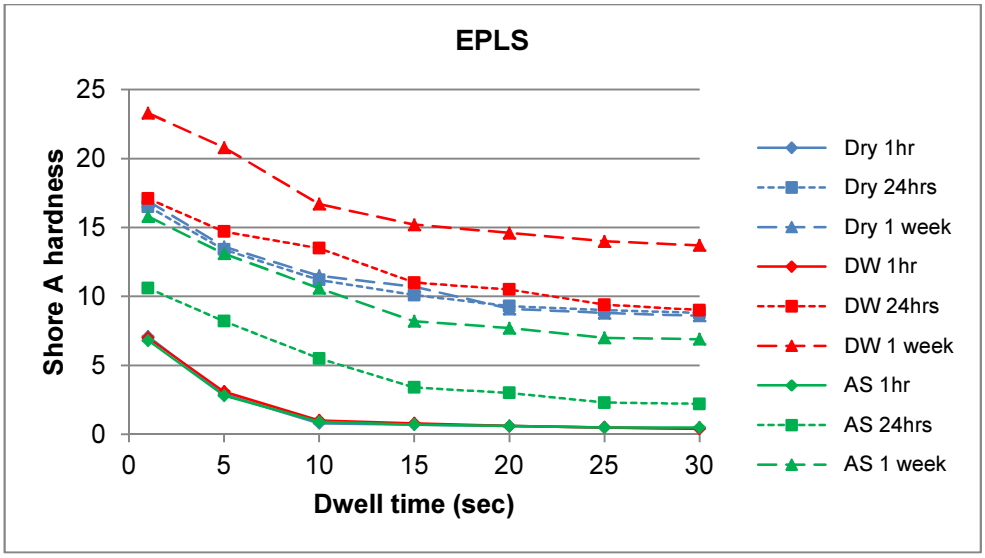
Mean (n=6) shore A hardness of CC 1.8 at different dwell times stored at 37°C in dry, DW and AS



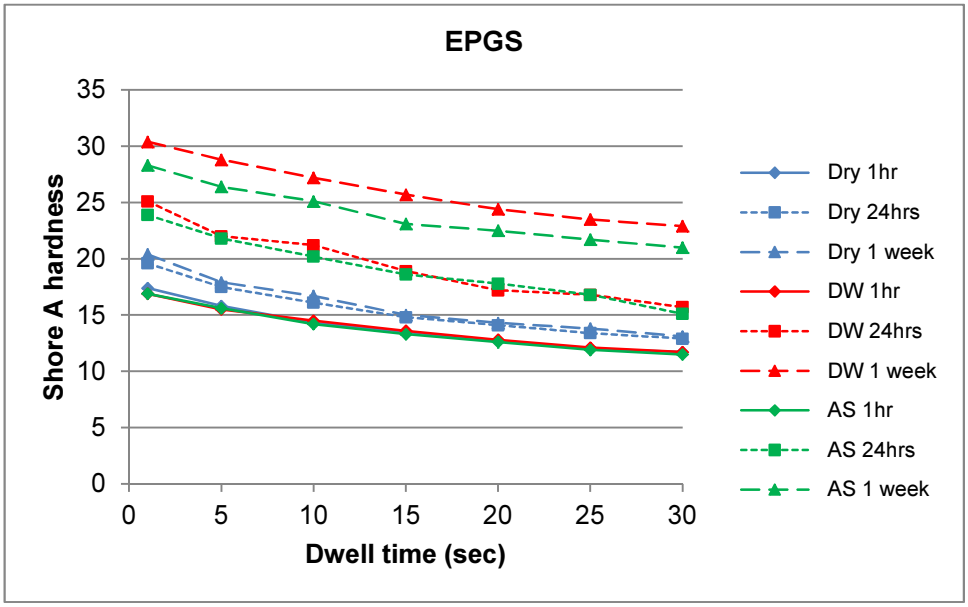
Mean (n=6) shore A hardness of VG 1.5 at different dwell times stored at 37°C in dry, DW and AS



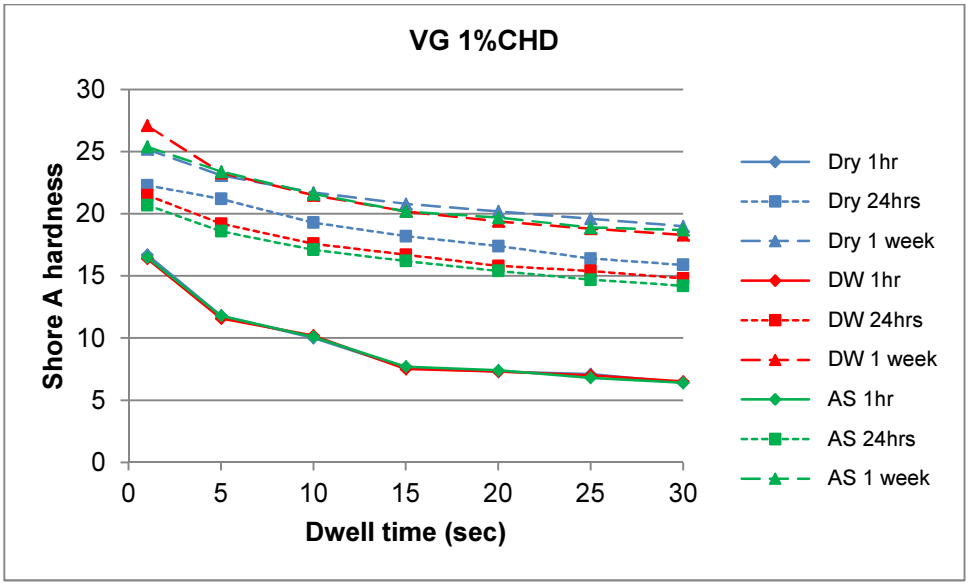
Mean (n=6) shore A hardness of VG 1.8 at different dwell times stored at 37°C in dry, DW and AS



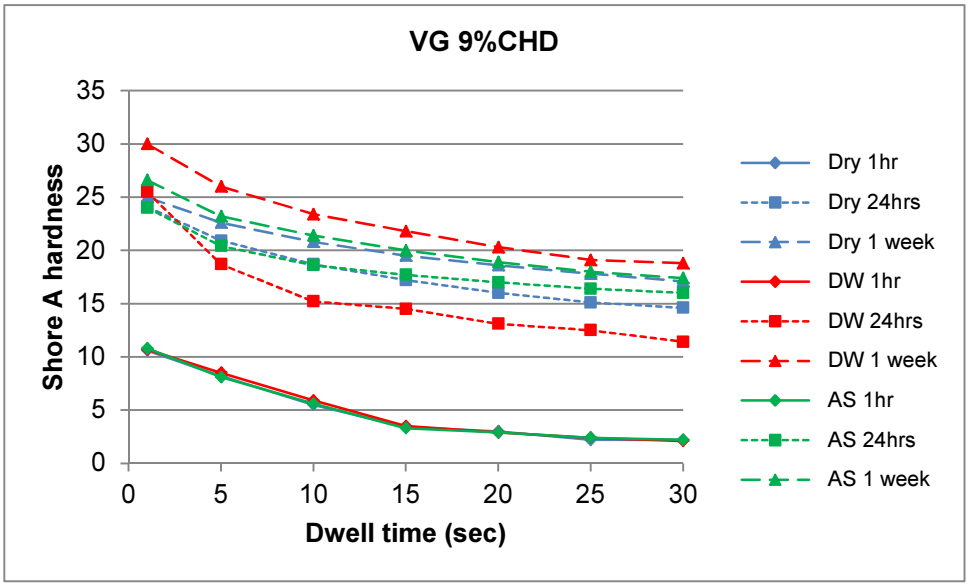
Mean (n=6) shore A hardness of EPLS at different dwell times stored at 37°C in dry, DW and AS



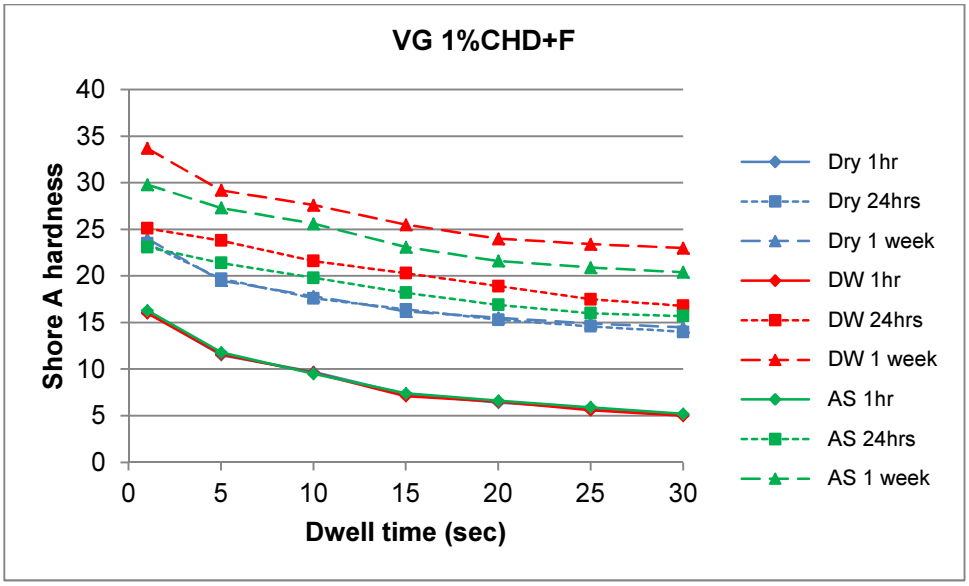
Mean (n=6) shore A hardness of EPGS at different dwell times stored at 37°C in dry, DW and AS



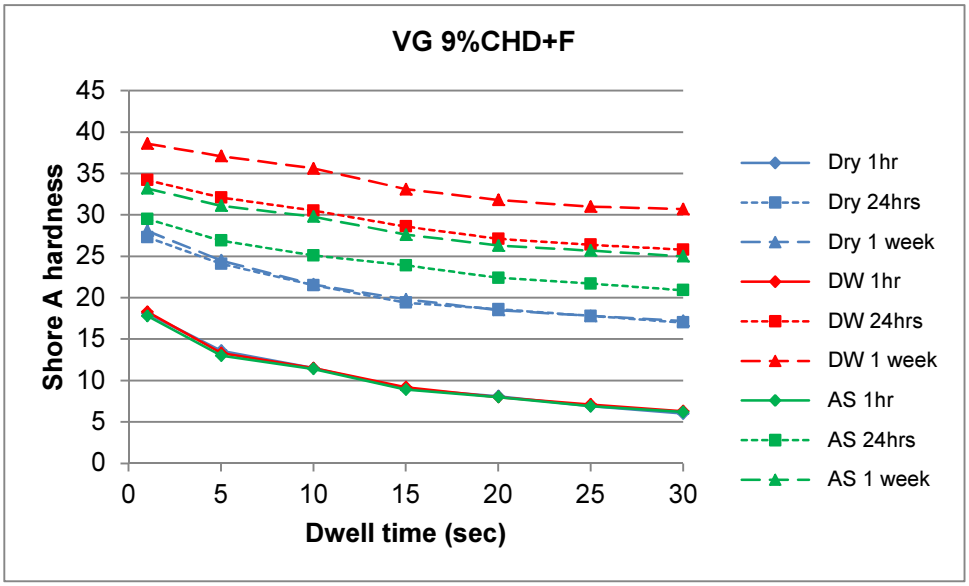
Mean (n=6) shore A hardness of VG 1%CHD at different dwell times stored at 37°C in dry, DW and AS



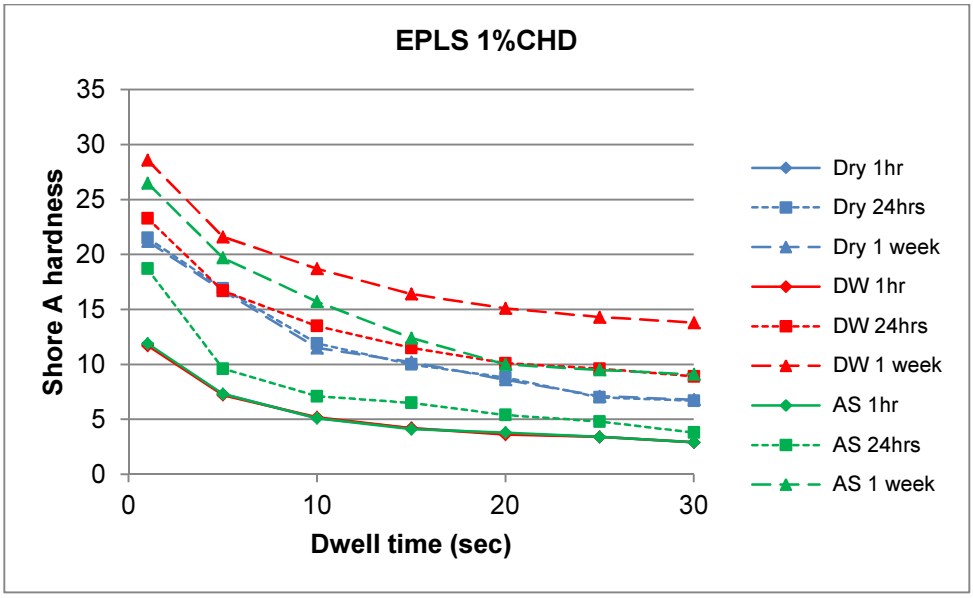
Mean (n=6) shore A hardness of VG 9%CHD at different dwell times stored at 37°C in dry, DW and AS



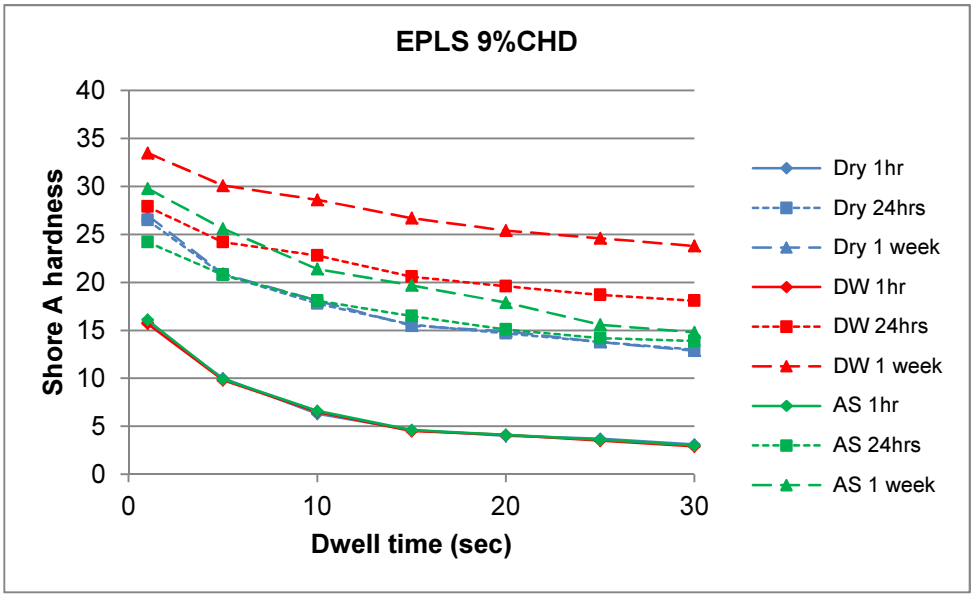
Mean (n=6) shore A hardness of VG 1%CHD+F at different dwell times stored at 37°C in dry, DW and AS



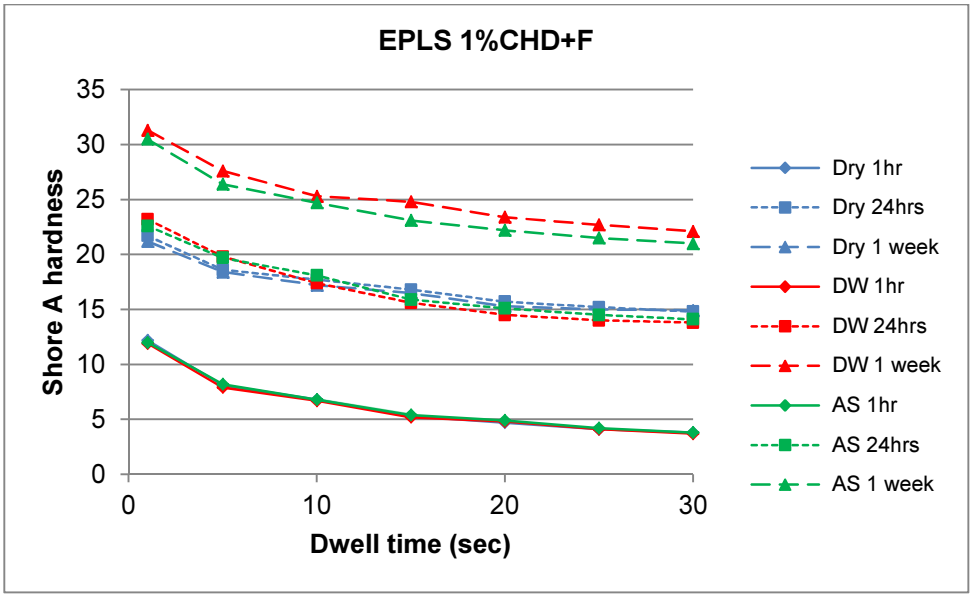
Mean (n=6) shore A hardness of VG 9%CHD+F at different dwell times stored at 37°C in dry, DW and AS



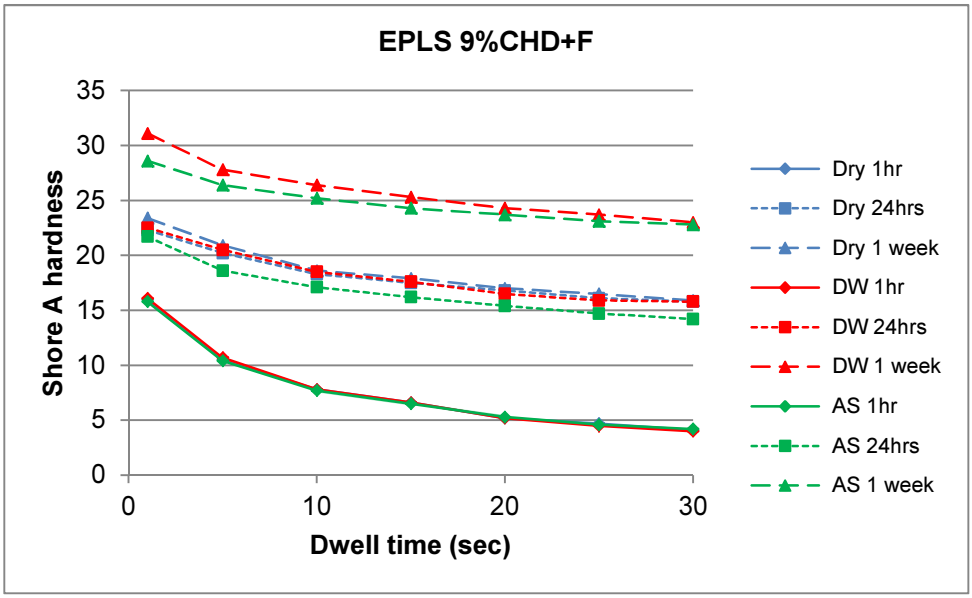
Mean (n=6) shore A hardness of EPLS 1%CHD at different dwell times stored at 37°C in dry, DW and AS



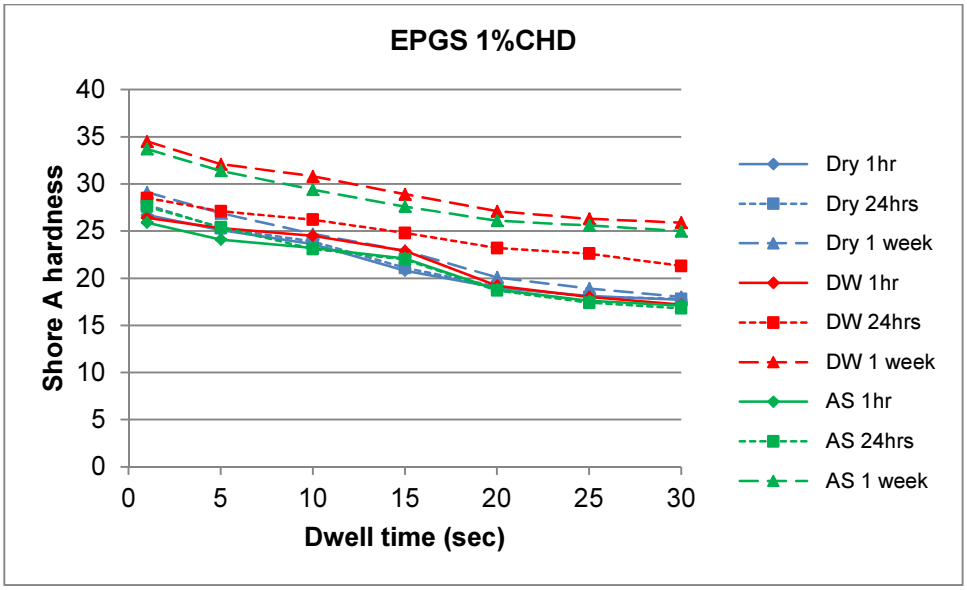
Mean (n=6) shore A hardness of EPLS 9%CHD at different dwell times stored at 37°C in dry, DW and AS



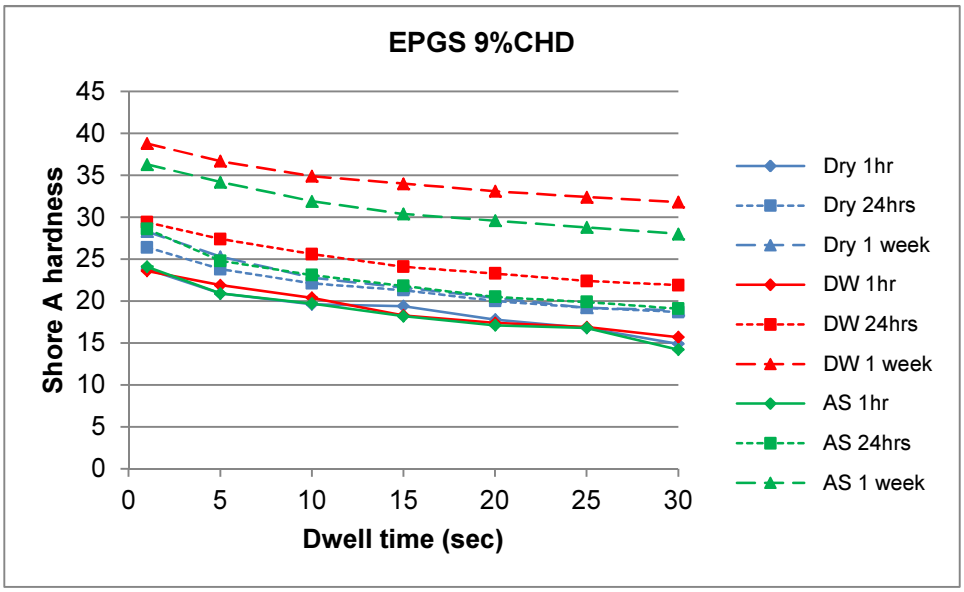
Mean (n=6) shore A hardness of EPLS 1%CHD+F at different dwell times stored at 37°C in dry, DW and AS



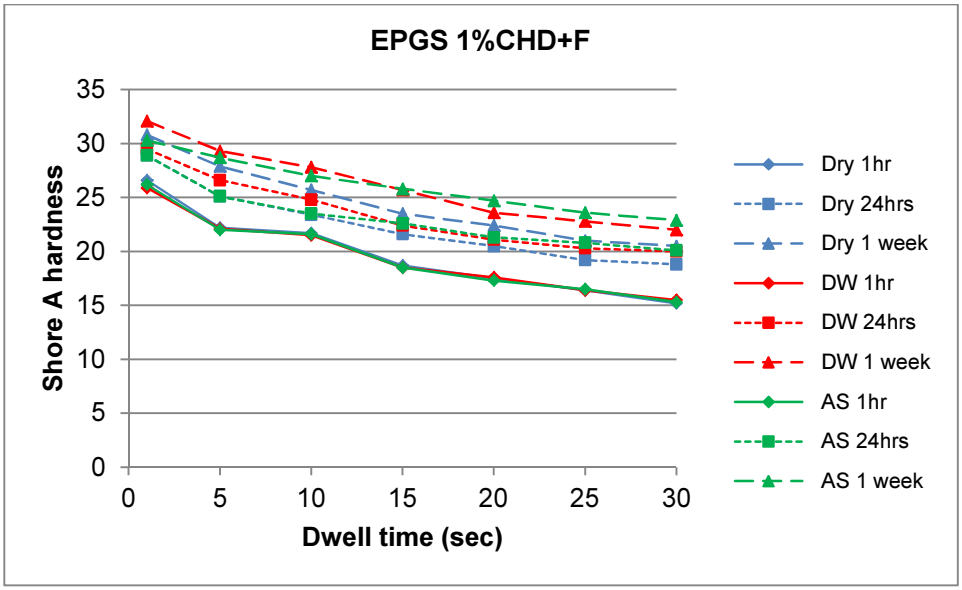
Mean (n=6) shore A hardness of EPLS 9%CHD+F at different dwell times stored at 37°C in dry, DW and AS



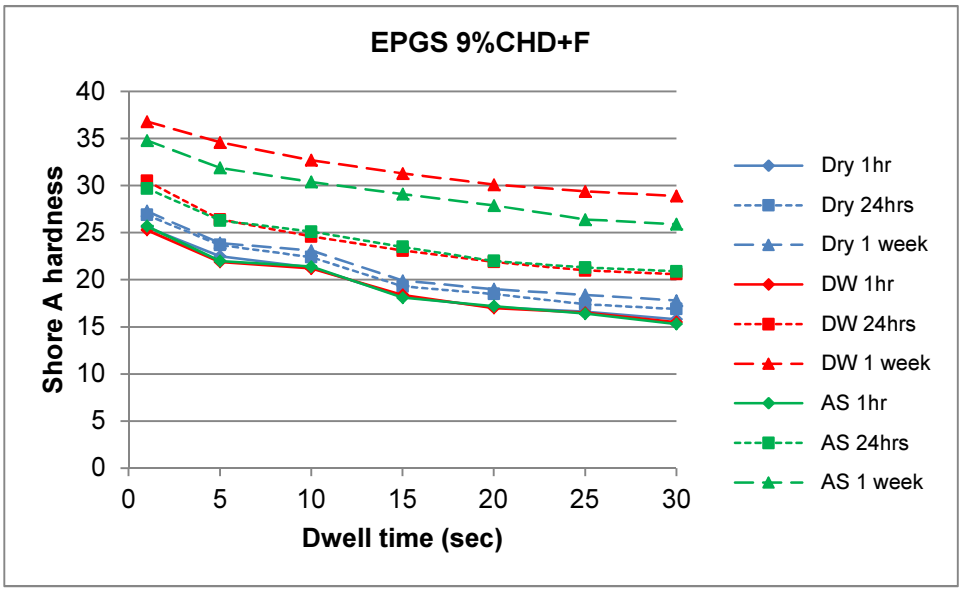
Mean (n=6) shore A hardness of EPGS 1%CHD at different dwell times stored at 37°C in dry, DW and AS



Mean (n=6) shore A hardness of EPGS 9%CHD at different dwell times stored at 37°C in dry, DW and AS

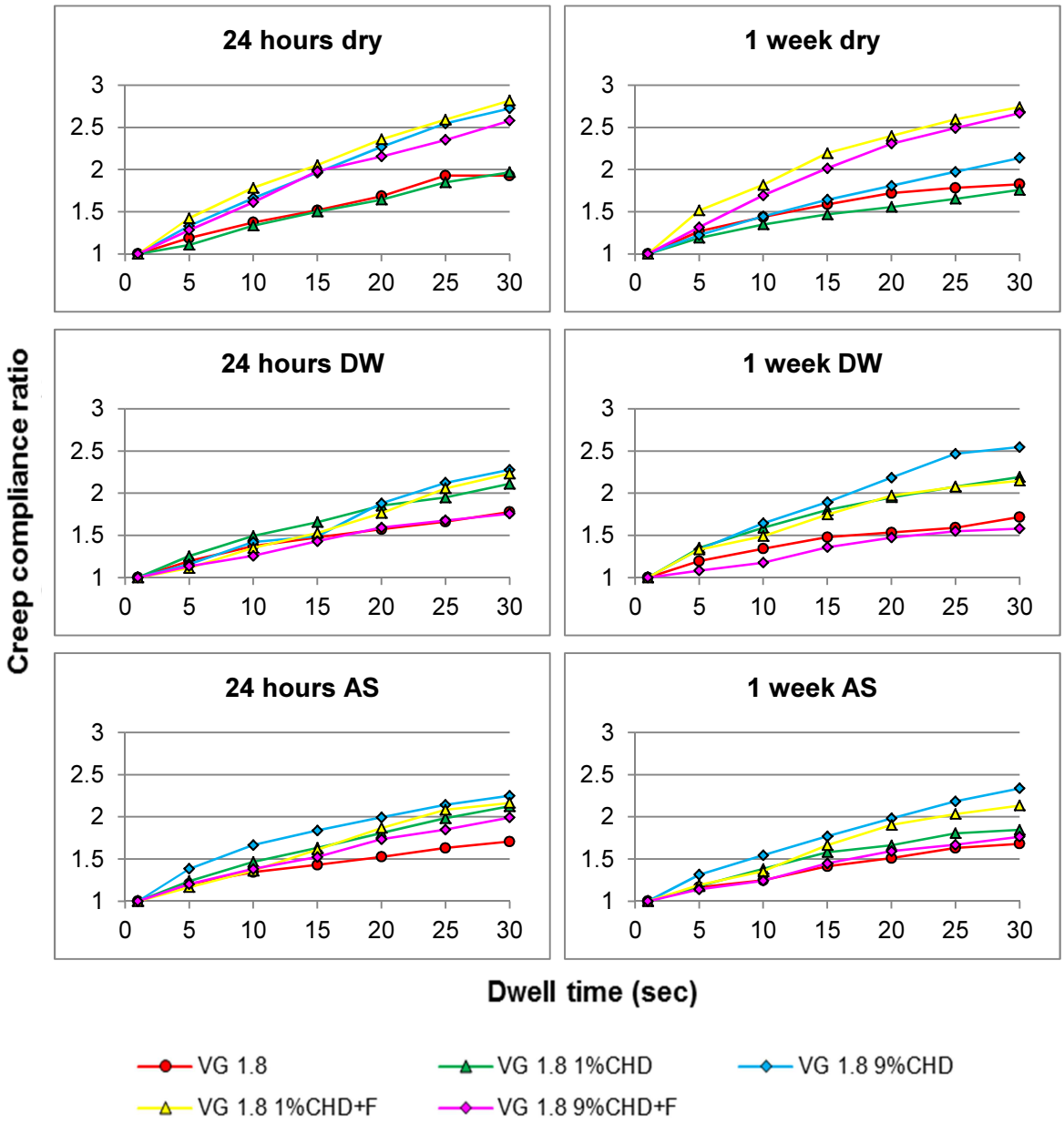


Mean (n=6) shore A hardness of EPGS 1%CHD+F at different dwell times stored at 37°C in dry, DW and AS

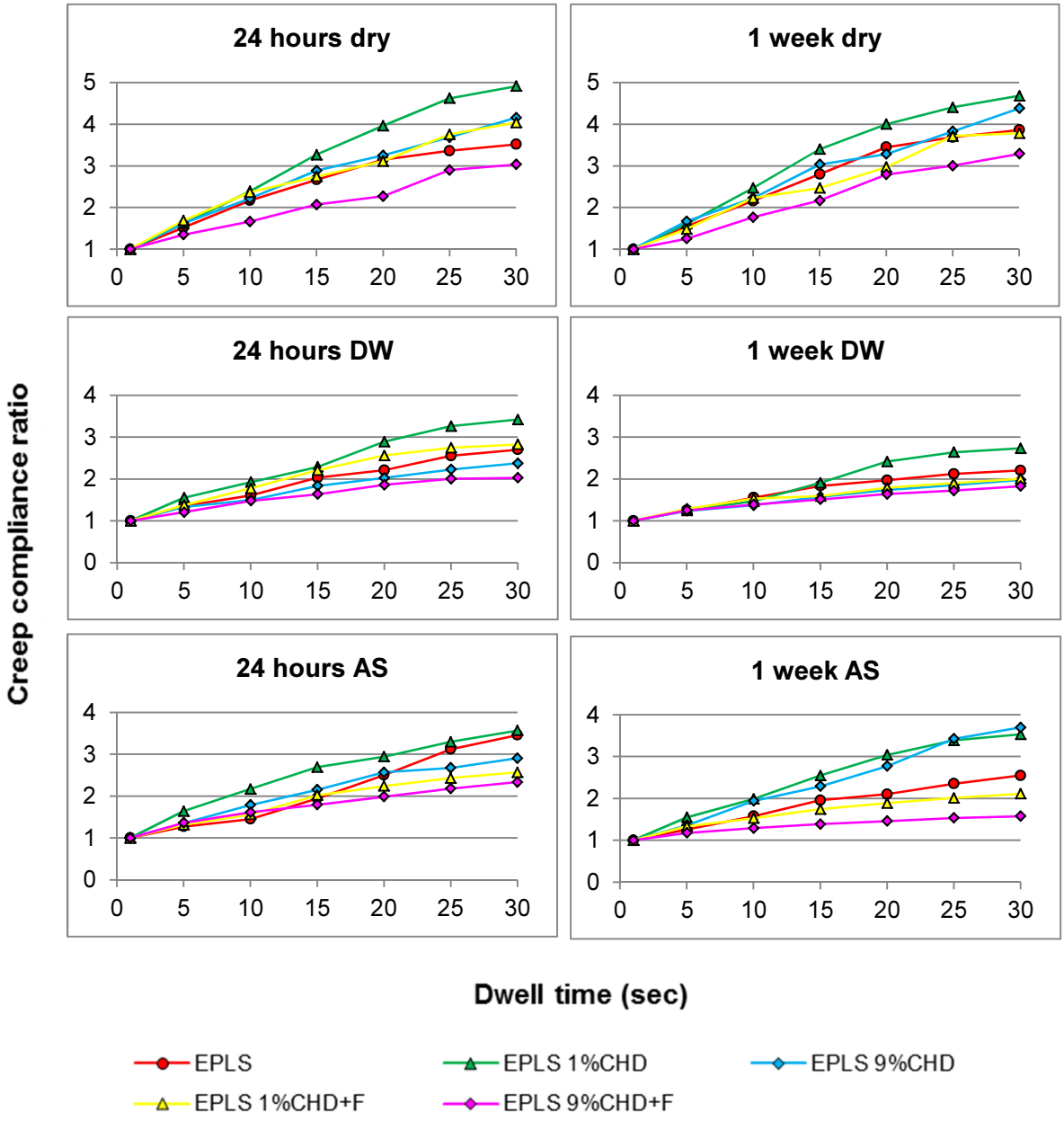


Mean (n=6) shore A hardness of EPGS 9%CHD+F at different dwell times stored at 37°C in dry, DW and AS

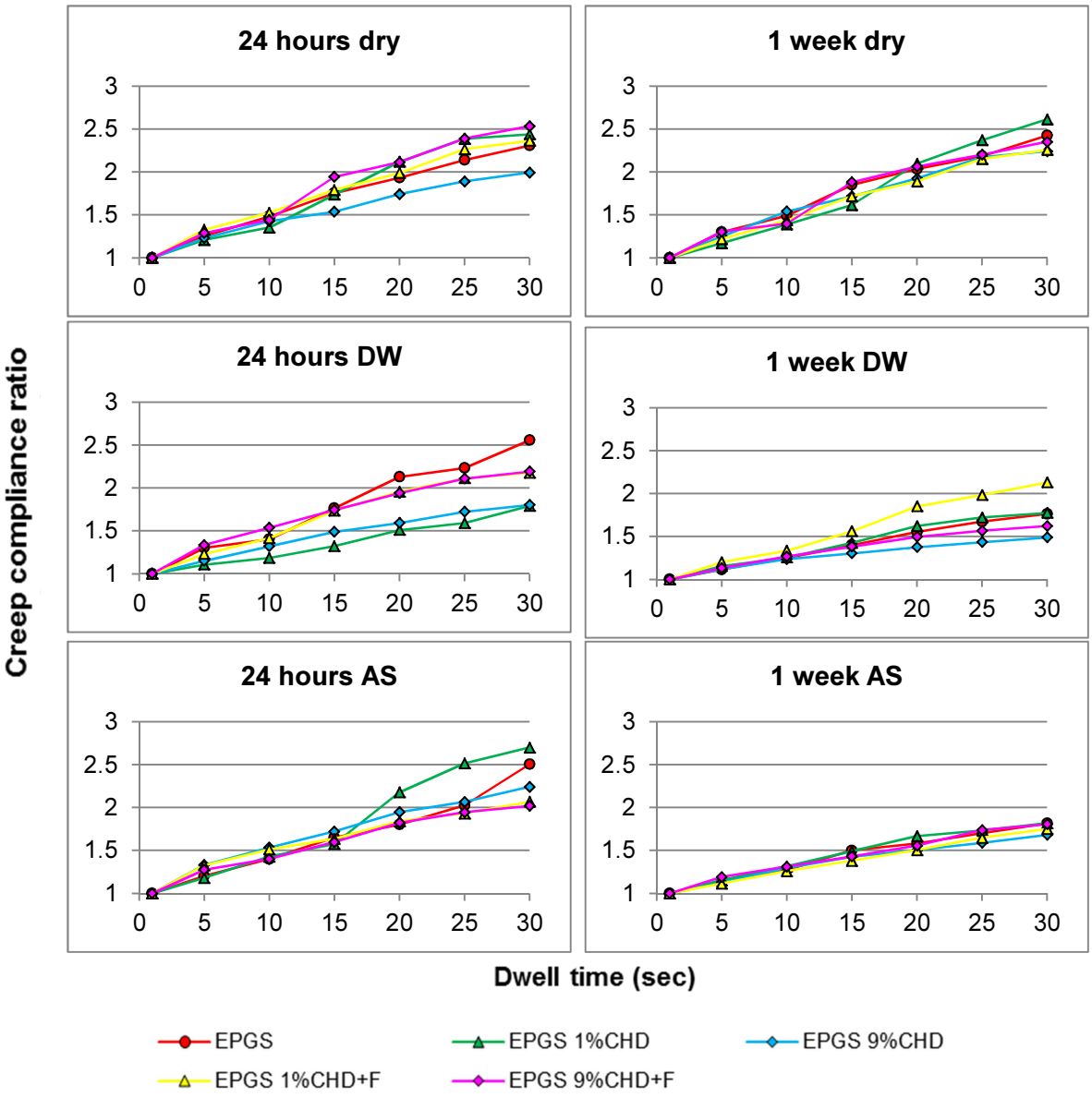
A4. Creep compliance ratio



Creep compliance ratio of VG, VG 1% and 9% CHD with and without 0.5% NaF stored dry, in DW and in AS at 37°C



Creep compliance ratio of EPLS, EPLS 1% and 9% CHD with and without 0.5% NaF stored dry, in DW and in AS at 37°C



Creep compliance ratio of EPGS, EPGS 1% and 9% CHD with and without 0.5% NaF stored dry, in DW and in AS at 37°C